Standard Guidelines
for the Environmental Monitoring of Chemicals

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I Monitoring Chemical Substances

I.1 Introduction

For the past few decades, industrialized societies have been both developing new chemical substances and producing large quantities of existing chemicals every year. These chemicals serve many useful purposes and are to be found in all aspects of modern life. They have contributed significantly, and continue to contribute significantly, to the improvement of human health, welfare and lifestyle. However, many chemicals are released into the environment during production, transportation, utilization, or disposal, and once there produce harmful side effects, particularly on human health and ecosystems, through the persistence of significant residues in the environment, or by bioaccumulation (bioconcentration) in food chains. Environmental pollution issues, such as organic mercury and PCB pollution, groundwater pollution with trichloroethylene and tetrachloroethylene, marine pollution by organic tin compounds, and dioxin emissions from garbage incineration, are not just scientific problems, but have also become significant social issues.

The recent increase in the number and quantity of chemicals produced has seen a concomitant increase in the range of related environmental problems. Environmental pollution by harmful chemicals is of concern not just to Japan, but also to many countries worldwide. At UNCED (the United Nations Conference on Environment and Development held in Rio de Janeiro in June 1992) it was determined in "Agenda 21" that the management of harmful chemicals in the environment is an international theme, and the IFCS (the International Forum of Chemical Safety) was established as an inter-governmental forum in an effort to provide active international follow-up to the Conference's resolutions.

In December 1994, concern over chemicals threatening the maintenance or preservation of the environment saw the formulation of Japan's "Basic Environment Plan." This Plan, based on the "Basic Environment Law" (November 1993) set out as policy that a knowledge of the environmental risk posed by chemicals was one of the basic requirements for environmental preservation. The Plan aims to both quantitatively evaluate environmental risk, and at the same time eliminate the risk by several means e.g. in order to decrease environmental risk during the production, use, and disposal of chemicals, the Plan set out (i) regulations for the

manner and methods of discharge of harmful chemicals, (ii) regulations for the management of chemical production and use appropriate to the degree of harmfulness (toxicity) of the chemical, (iii) regulations for the development and spread of substitute technology and products, and (iv) regulations for the appropriate treatment of recovered harmful chemicals.

In order to be able to control and manage appropriately the environmental risk posed by chemicals (decrease the risk), it is first important to determine and quantitatively evaluate the environmental risk. Such risk evaluation is based on a chemical "risk assessment" (an evaluation of the toxicity of the chemicals in question to humans or ecosystems) and an "exposure assessment" (an evaluation of the degree of chemical exposure experienced by humans or ecosystems based on determinations of the environmental concentrations of the chemicals).

After passing the Resolution accompanying the Law Concerning the Examination and Regulation of Manufacture etc. of Chemical Substances (the Chemical Substances Control Law) in 1973, the Japanese Parliament (the Diet) amended the law to make the national government perform a safety check of chemicals produced in Japan or imported, and in use at that time (of which over 20,000 were listed). This was followed by the Environmental Agency beginning a survey of residues of these chemicals in the general environmental. Later the objectives of these investigations were expanded to include newly registered chemicals and unintentionally produced chemicals (by-products or contaminants). While the Chemical Substances Control Law, by controlling production (or import) and regulating the use of harmful chemicals to control their environmental toxicity and residues, is an important method for environmental risk management, investigations into the actual levels of environmental contamination by chemicals provides the type of important basic information required for exposure evaluation. Furthermore, such investigations provide, and are expected to provide, information required in the performance of other environmental risk management functions, such as discharge regulation. The Chemical Substances Control Law was enacted in October 1973 amid concern over environmental pollution by PCBs, and enforced from April 1974. According to the Chemical Substances Control Law new chemicals were to be investigated before production or import (pre-examination of new chemicals) if the chemicals were (i) chemically inert under natural conditions, (ii) if they were easily accumulated in the living tissue (high bio-accumulation potential), (iii) if they have posed a significant risk to human health when ingested / absorbed on a continuous basis (chronic toxicity), and (iv) to be registered as 'first grade' and the production, import, and use etc. regulated, specified chemicals having all these characteristics. By the end of December 1995, 5,879 new chemicals (4,404 produced locally, 1,475 imported) were reported, of which the safety of 4,679 chemicals (3,591 produced locally,

1,088 imported) had been investigated.

Existing chemicals are investigated by three government organizations : chemical decomposition by microorganisms and bioaccumulation in fish and other marine products is investigated by the Ministry of International Trade and Industry (MITI), human toxicity is investigated by the Ministry of Health and Welfare, and general investigations into actual environmental residue levels and ecological effects are performed by the Environmental Agency. By December 1996, nine chemicals had been designated as Class 1 Specified Chemical Substances - PCBs, HCBs, PCNs, aldrin, dieldrin, endrin, DDT, chlordanes, and bis(tributyl tin)=oxide.

The Chemical Substances Control Law was revised in May 1986 (enforced from April 1987) after detection of trichloroethylene contamination of groundwater in Japan. This revision covered the production and import of Designated Chemical Substances which do not significantly bioaccumulate, are slightly degradable and have indeterminate chronic toxic effects. Where there are strong concerns over damage to human health caused by environmental contamination by such chemicals, the chemical manufacturers were required to investigate and report chemical toxicity data. If such chemicals showed significant toxicity, their production and import was regulated. To date 257 chemicals, such as chloroform, 1,2-dichloroethane, have designated as Designated Chemical Substances. Since April 1989, when tetrachloromethane, tetrachloroethylene, and trichloroethylene were designated Class 2 Specified Chemical Substances, there are now 23 such Class 2 Specified Chemical Substances.

In 1974, after enactment of the Chemical Substances Control Law, the Environmental Agency began the Survey for Chemical Substances (determining the real state of the environment), and other related projects. In order to systematically investigate the large number of chemicals, a temporary toxic substances list was prepared based on known human health effects. Chemicals on the list and which received priority for investigation were those which (i) are highly toxic and are regulated under the Law, (ii) which are known to be difficult to degrade (persistent chemicals), and (iii) which have structures similar to PCBs, or are industrial substitutes for such chemicals.

As the number and types of matrices under investigation have increased, new chemical analytical methods have had to be developed for most chemicals. As a result, since 1977 research has been classified into three areas, namely the development of chemical analytical methods, general environmental research, and specific highly detailed environmental research

projects. Research bodies were organized with local government environmental research institutes to these research areas.

As such research was systematized, the first general investigation of chemical environmental safety was conducted from 1979 to 1988 (first 10 Year Plan) using as its starting point the above mentioned temporary toxic substances list. Since 1988, a second general investigation of chemicals environmental safety has been conducted (second 10 Year Plan). Hereafter follows a summary of chemical monitoring in Japan.

I.2 Monitoring - Concepts and Design

Environmental management involves three basic types of activities. First, measurement and observation to describe both the current state of the environment and any change. Second, evaluation and analysis of environmental data. Third, developing warning systems based on predetermined standards to alert scoety to change. "Monitoring" in this manual includes the first activity (scientifically planned measurement and observation systems) and also partly includes the second action (evaluation).

Many chemicals discharged into atmosphere, water, and onto farmland are spread all over the planet. These chemicals are now also found pristine terrestrial and ocean environments far from human habitation. Some of them are known to be highly toxic at high doses, but it is unknown if such chemicals have long term effects on people and other living things when the doses are small. In addition, once discharged these harmful chemicals exist in the environment without degradation, or with only minimal degradation for tens of years, and it is clear that some animals and plants have been affected by these harmful chemicals to the point of population decrease or increase in diseases.

Heavy metals (lead, mercury, cadmium), organochlorine compounds (DDT and its metabolites and degradation products, PCB), and petroleum products are often cited as 'slightly degradable' harmful chemicals. Pollution occurs in the every media - atmospheric, aqueous, soil, and in organisms. However, it is important that these products stay long in the biosphere such as soil and seawater. Almost every kinds of artificial pollutants end up in the ocean. Industrial wastes and pesticides are discharged directly into coastal waters, or are carried into the sea

through rivers. Huge amounts of harmful chemicals are often dumped onto bottom of the sea or into the ocean far from the coast. Pollutants carried through by the atmosphere are deposited by rain or absorbed directly onto the surface of the ocean.

The biosphere has therefore effectively become a toxic waste dump, and that inevitably has effects on the growth and propagation of animals and plants. Every kinds of pollutants affects on ecology structure by decreasing species diversity though there are level differences. Harmful chemicals endanger human health directly or accumulated through food chain. It is unknown if the distribution pattern of these chemicals is stable (quasi-equilibrium state) at moment, or if some of these chemicals will keep circulating in the biosphere following transport processes which are not well understood when chemical discharge from urban, industrial, and agricultural activity ceases.

Observing varied bioinfluences makes clear about effects which pollutants give biota. For example, change of biomass and the sphere of distribution, change of group structure of animals and plants, total exchange of ecosystem or change of productivity are given. Therefore, choosing and evaluating specified variety of living thing or items to describe high level change of creature system can make special or general effects giving biota clear.

It is necessary to consider constructing automatic adjusting system as monitoring for check chemical influences on animals and plants and humans circulating the environment where they are living. It is needed to make priority from several 10,000 chemicals used commercially. Also it is decided which environmental media (atmosphere, water soil, and living things) tends to include these chemicals depending on the physical, chemical character of these chemicals. It is important to select environmental media to meet the chemicals. Concentration of pollutants in the atmosphere changes depending on the climate condition. So it is needed to sample considering space and time wise. Therefore the total monitoring system has to be planned highly sensitively which includes these special sub-program needs.

I.3 Target Chemicals

In order to determine environmental chemical residues, it is first necessary to choose target chemicals from among the many thousands currently in commercial use. The Environment

Agency in Japan has been developing chemical analytical methods for more than 700 chemicals and accumulating environmental pollution data for the past 20 years (details of the analytical methods are described in subsequent chapters. All such analytical methods and other information will be useful for other countries monitoring chemicals in their environment. A list of the chemicals is shown in **Table I-2**). The number of chemicals which have been found in water, sediments, in fish, and in the air between 1974 and 1995 is summarized in **Table I-1**.

Table I-1. Summary of number of chemicals detected in the environmental survey (1974 - 1995).

	Water	Sediment	Fish	Air	Total
No. of chemicals investigated	731	710	226	174	752
No. of chemicals detected	135	219	87	104	287
Detection rate (%)	18.5	30.8	38.5	59.8	38.2

Table I-2. Summary of the results of environmental survey for chemical substances 1974 to 1994 (conducted by Environmental Agency)

○ : Detection / × : Undetection
W:Water S:Sediment F:Fishes O:Others(A:Air R:Rain Water P:Plankton)

Substance	CAS No.	Year	W	S	F	O
Acenaphthene	83-32-9	83	×	○		
		84	○	○	○	
Acenaphthylene	208-96-8	83	×	○		
		84	○	○	○	
Acephate	30560-19-1	93	×	×	×	
Acetaldehyde	75-07-0	77	×	○		
		87	×			A○
Acetonitrile	75-05-8	77	×	×		
		87	×	○		A○
		91				A○
		92	○	○		
Acrolein	107-02-8	78	×	×		
		87	×			A×
Acrylamide	79-06-1	75	×			
		91	○	○	×	
Acrylonitrile	107-13-1	77	×	×		
		87	×	○		A○
		91				A○
		92	×	○	×	
Adipic acid	124-04-9	85	×	○		
Adiponitrile	111-69-3	78	×	×		
Aldrin	309-00-2	74	×	×	×	
Alkyldimethylbenzyl-ammonium chloride (*1) (Benzalkonium chloride)	8001-54-5 68391-01-5	82	×	○		
		83	×	○	×	
Allylamine	107-11-9	81	×	×		
Allyl chloride	107-05-1	77	×			
1-Aminoanthraquinone	82-45-1	85	×	○		
2-Aminoanthraquinone	117-79-3	85	×	×		
3-Aminobenzenesulfonic acid		81	×	×		
o-Aminobiphenyl	90-41-5	77	×	×		
2-Amino-5-chloro-4-methylbenzenesulfonic acid	88-53-9	80	×	×		
1-Amino-2-methoxy-5-methylbenzene	120-71-8	85	×	×		
1-Amino-2-methylanthraquinone	82-28-0	86	×	×		
1-Aminonaphthalene-4-sulfonic acid	84-86-6	85	×	×		
2-Aminonaphthalene-1-sulfonic acid	81-16-3	85	×	×		

Substance	CAS No.	Year	W	S	F	O
2-Aminonaphthalene-5-sulfonic acid	81-05-0	85	×	×		
2-Aminonaphthalene-6-sulfonic acid	93-005	85	×	×		
2-Aminonaphthalene-7-sulfonic acid	494-44-0	85	×	×		
2-Aminonaphthalene-8-sulfonic acid	86-60-2	85	×	×		
1-Amino-8-naphthol-3, 6-disulfonic acid	90-20-0	80	×	×		
2-Amino-5-naphthol-7-sulfonic acid	87-02-5	80	×	×		
o-Aminophenol	95-55-6	86	×	×		
m-Aminophenol	591-27-5	86	○	×		
p-Aminophenol	123-30-8	86	×	×		
2-Aminopyridine	504-29-0	83	×	×		
3-Aminopyridine	462-08-8	83	×	×		
4-Aminopyridine	504-24-5	83	×	×		
3-Amino-1, 2, 4-triazole	61-82-5	84	×	×		
Aniline	62-53-3	76	○	○		
		90	○	○	○	A○
Anionic surfactants		74	○			
o-Anisidine	90-04-0	76	○	○		
		90	○	○	×	A×
m-Anisidine	536-90-3	76	○	○		
		90	○	×	○	A×
p-Anisidine	104-94-9	76	○	○		
		90	×	×	×	A×
Anthracene	120-12-7	76	×	○		
		77	×	○		
Anthraquinone	84-65-1	88	×	○		
		89	×	○		
Atrazine (2-Chloro-4-ethylamino-6-isopropyl-amino-*sym*-triazine)	1912-24-9	91	×	×		
Auramine	2465-27-2	86	×	×		
Azinphos-methyl	86-50-0	93				A×
Azobisisobutyronitrile	78-67-1	79	×	×		
C. I. Basic Green 4	569-64-2	85	×	×		
C. I. Basic Violet 10	81-88-9	86	×	×		
Benzaldehyde	100-52-7	84	×	○		

(*1) Total of compounds with alkyl chains having 12, 14 or 16 carbons

I Monitoring Chemical Substances

Substance	CAS No.	Year	W	S	F	O
Bentazone [3-Isopropyl-1H-2, 1, 3-benzothiadiazin-4 (3H)-one-2, 2-dioxide]	25057-89-0	92	○	×	×	
Benthiocarb (S-4-Chlorobenzyl N, N-diethylthiocarbamate)	28249-77-6	92	×	○	×	A○
Benz [a] anthracene	56-55-3	89	×	○	○	A○
Benzene	71-43-2	77	×	×		
		85	○	○		
		86	○	○	○	
Benzene tricarboxylic acid tris (2-ethylhexyl) ester	3319-31-1	80	×	×		
Benzidine	92-87-5	77	×	×		
1, 4-Benzodinitrile	623-26-7	81	×	×		
Benzoepin (Hexachloro-hexahydromethano-benzodioxathepin oxide) (α-, β-) (Endosulfan)	959-98-8 33213-65-9	82	×	×		
		92				A×
Benzo [b] fluoranthene Benzo [j] fluoranthene Benzo [k] fluoranthene (*2)	205-82-3 205-99-2 207-08-9	89	×	○	○	A○
Benzoic acid	65-85-0	85	○	○		
		86	○	○	○	
Benzonitrile	100-47-0	77	×	×		
Benzo [ghi] perylene	191-24-2	89	○	○	○	A○
Benzophenone	119-61-9	81	×	×		
Benzo [a] pyrene	50-32-8	89	×	○	○	A○
Benzo [e] pyrene	192-97-2	89	×	○	×	A○
Benzothiazole	95-16-9	83	×	○		
Benzyl alcohol	100-51-6	85	×	○		
Benzyl butyl phthalate	85-68-7	85	×	○		
Benzyl chloride	100-44-7	76	×	×	×	
		77	×	○		
		89	×	×		A○
Biphenyl	92-52-4	76	×	×	×	
Biphenyl ether	101-84-8	76	×	×	×	
		84	×	×		
Bis (4-bromophenyl) ether	2050-47-7	84	×	×		
1, 1-Bis (t-butylperoxy)-3, 3, 5-trimethyl-cyclohexane	6731-36-8	89	×	○	×	
Bis (2-chloroethyl) ether	111-44-4	77	×	×		
		84	×	×		
Bis (2-chloroisopropyl) ether	108-60-1	84	×	×		
1, 1-Bis (p-chlorophenyl)-2, 2, 2-trichloroethanol	115-32-2	78	×	×		

Substance	CAS No.	Year	W	S	F	O
4, 4'-Bis (dimethylamino) benzophenone	90-94-8	85	×	×		
4, 4'-Bis (dimethyl-aminophenyl) methane	101-61-1	86	×	×		
2, 2-Bis [4-(2-hydroxy)-3, 5-dibromophenyl] propane	4162-45-2	86	○	×		
2, 2-Bis (4-hydroxy-phenyl) propane (Bisphenol A)	80-05-7	76	×	×	×	
Bis (2, 3, 3, 3-tetrachloropropyl) ether	127-90-2	81	×	×		
		84	×	×		
Bis (tribromophenoxy) ethane	37853-59-1	87	×	○	×	
BPMC (o-s-Buthylphenyl methylcarbamate)	3766-81-2	88	×	×		A○
o-Bromoaniline	615-36-1	84	×	×		
m-Bromoaniline	591-19-5	84	×	×		
p-Bromoaniline	106-40-1	84	×	×		
Bromobenzene	108-86-1	81	×	×		
1-Bromobutane	109-65-9	81	×	×		
Bromochloromethane	74-97-5	76	×	×	×	
o-Bromophenol	95-56-7	83	×	×		
m-Bromophenol	591-20-8	83	×	×		
p-Bromophenol	106-41-2	83	×	○		
4-Bromophenylphenyl ether	101-55-3	84	×	×		
1-Bromopropane	106-94-5	81	×	×		
β-Bromostyrene	103-64-0	85	×	×		
Butachlor	23184-66-9	94	×	×	×	
Butadiene	106-99-0	77	×	×		
1, 3-Butanediol	107-88-0	86	×	×		
1, 4-Butanediol	110-63-4	86	×	×		
n-Butanol	71-36-3	79	×	×		
s-Butanol	78-92-2	79	×	×		
t-Butanol	75-65-0	79	×	×		
1-n-Butoxy-2, 3-epoxy-propane	2426-08-6	84	×	×		
2-Butoxyethanol	111-76-2	76	×	×		
Butyl acrylate	141-32-2	80	×	×		
n-Butylamine	109-73-9	81	×	×		
p-t-Butylbenzoic acid	98-73-7	85	×	○		
		86	○	○	○	
2-t-Butyl-4-(2, 4-dichloro-5-isopropoxy-phenyl)-1, 3, 4-oxadiazolin-5-one	19666-30-9	81	×	×		
n-Butyl formate	592-84-7	81	×	×		
i-Butyl formate	542-55-2	81	×	×		
t-Butylhydroxyanisole (BHA)	121-00-6	80	×	×		

(*2) The values are the total of the three compounds.

Substance	CAS No.	Year	W	S	F	O
p-t-Butylhydroxyphenol	1948-33-0	80	×	×		
4, 4'-Butylidene bis (6-t-butyl-3-methyl phenol)	85-60-9	81	×	×		
Butyl methacrylate	97-88-1	79	×	×		
Butylnaphthalene-sulfonic acid	25638-17-9	81	×	×		
p-t-Butylphenol	98-54-4	76	×	×		
ε-Caprolactam	105-60-2	77	×	○		
		91	×	×	○	A○
Captafol [cis-N-(1, 1, 2, 2-Tetrachloroethylthio)-4-cyclohexene-1, 2-carboximide]	2425-06-1	80	×	×		
Carbazole	86-74-8	76	×	×		
		94				A×
Carbofuran (2, 3-Dihydro-2, 2-dimethyl-benzofuran-7-yl methylcarbamate)	1563-66-2	92	×	×	×	
Carbon disulfide	75-15-0	77	×	×		
		92				A○
Carbon tetrachloride	56-23-5	74	×			R○
		75	○			R○
		79				A○
		80				A○
		83				A○
cis-Chlordane	57-74-9	82	×	○	○	
		86				A○
trans-Chlordane	57-74-9	82	×	○	○	
		86				A○
γ-Chlordene	3734-48-3	82	×	○	○	
		86				A○
Chlormethoxynil (2, 4-Dichlorophenyl-3'-methoxy-4'-nitrophenyl ether)	32861-85-1	82	○	×		
		91	×	×		A×
Chlorinated paraffines	63449-39-8	79	×	○		
		80	×	○	×	
Chloroacetaldehyde	107-20-0	80	×	×		
Chloroacetic acid	79-11-8	84	○	○		
Chloroacetone	78-95-5	86	×	×		
o-Chloroaniline	95-51-2	76	○	○	×	
		90	○	○	○	A×
m-Chloroaniline	108-42-9	76	○	○	×	
		90	○	○	×	A×
p-Chloroaniline	106-47-8	76	○	○	×	
		90	×	○	×	A×
1-Chloroanthraquinone	82-44-0	85	×	×		
2-Chloroanthraquinone	131-09-9	85	×	×		
o-Chlorobenzaldehyde	89-98-5	84	×	×		

Substance	CAS No.	Year	W	S	F	O
m-Chlorobenzaldehyde	587-04-2	84	×	×		
p-Chlorobenzaldehyde	104-88-1	84	×	×		
Chlorobenzene	108-90-7	76	×	×	×	
		83				A○
o-Chlorobenzoic acid	118-91-2	85	×	×		
Chlorocyclohexane	542-18-7	77	×	×		
Chlorodibromomethane	124-48-1	80				A○
		81	○	○		
		83				A○
1-Chloro-2, 4-dinitrobenzene	97-00-7	78	×	×		
2-Chloroethyl vinyl ether	110-75-8	84	×	×		
2-Chloro-6-methylaniline	87-63-8	81	×	×		
3-Chloro-4-methylaniline	95-74-9	81	×	×		
4-Chloro-2-methylaniline	95-69-2	81	×	×		
2-Chloro-5-methylphenol	615-74-7	84	×	×		
2-Chloro-6-methylphenol	87-64-9	84	×	×		
4-Chloro-2-methylphenol	1570-64-5	84	×	×		
4-Chloro-3-methylphenol	59-50-7	84	×	×		
1-Chloro-2-methyl-propane	513-37-1	80	×	×		
3-Chloro-2-methyl-propene	563-47-3	80	×	×		
1-Chloronaphthalene	90-13-1	77	×	×		
		86	×	×		
2-Chloronaphthalene	91-58-7	77	×	×		
		86	×	×		
4-Chloro-2-nitroaniline	89-63-4	78	×	×		
o-Chloronitrobenzene	88-73-3	75	×			
		91	×	×	×	A○
m-Chloronitrobenzene	121-73-3	75	×			
		94	×	×	×	A×
p-Chloronitrobenzene	100-00-5	78	×	×		
		91	×	×	×	A○
2-Chloro-5-nitrobenzene-sulfonic acid	96-73-1	79	×	×		
Chloronitrofen (2, 4, 6-Trichlorophenyl-4'-nitrophenyl ether)	1836-77-7	78	×	×		
		82	○	○		
		91	×	×		A×
4-Chloro-3-nitro-α, α, α-trifluorotoluene	121-17-5	81	×	×		
Chloropentabromo-cyclohexane	87-84-3	85	×	×		
o-Chlorophenol	95-57-8	78	×	×		
m-Chlorophenol	108-43-0	78	×	×		
p-Chlorophenol	106-48-9	78	×	×		
Chloropicrin	76-06-2	79	×	×		
		94	×			A×
Chloroprene	126-99-8	77	×			

I Monitoring Chemical Substances

Substance	CAS No.	Year	W	S	F	O
1-Chloropropane	540-54-5	81	×	×		
2-Chloropropane	75-29-6	81	×	×		
2-Chloropyridine	109-09-1	80	×	×		
o-Chlorostyrene	2039-87-4	81	×	×		
m-Chlorostyrene	2039-85-2	81	×	×		
p-Chlorostyrene	1073-67-2	81	×	×		
Chlorothalonil (Tetrachloroisophthalo-nitrile)	1897-45-6	77	×	×		
		91	×	×	×	A×
o-Chlorotoluene	95-49-8	79	×	×		
		89	×	×		A○
p-Chlorotoluene	106-43-4	79	×	×		
		89	×	×		A×
Chlorpyrifos [O, O-Diethyl-O-(3, 5, 6-trichloro-2-piridyl) phosphorothioate]	2921-88-2	83	×	×		
		88	×	○	×	A×
		90	×	○		
o-Cresol	95-48-7	77	×	×		
m-Cresol	108-39-4	77	×	×		
p-Cresol	106-44-5	77	×	○		
Cresyldiphenyl phosphate	26444-49-5	81	×	×		
Crotonaldehyde	4170-30-3	87	×			A×
CVMP [2-Chloro-1-(2, 4, 5-trichlorophenyl) vinyl dimethyl phosphate]	961-11-5	88	×	×	×	A×
CVP [2-Chloro-1-(2, 4-dichlorophenyl) vinyl-diethyl phosphate]	470-90-6	88	×	○	×	A×
		93 (*3)	×	×	×	
			×	×	×	
2-Cyanopyridine	100-70-9	84	×	×		
3-Cyanopyridine	100-54-9	84	×	×		
4-Cyanopyridine	100-48-1	84	×	×		
Cyclohexane	110-82-7	79	×	×		
Cyclohexanone	108-94-1	80	×	×		
Cyclohexylamine	108-91-8	82	○	○		
		83	○	○	○	
N-Cyclohexyl-2-benzo-thiazolesulfenamide	95-33-0	77	×	×		
Cyclopentadiene	542-92-7	80	○	×		
Cyclopentane	287-92-3	80	○	○		
pp'-DDD (p, p'-Dichloro-diphenyldichloroethane)	72-54-8	74	×	○	○	
pp'-DDE (p, p'-Dichloro-diphenylethylene)	72-55-9	74	×	○	○	
op'-DDT (o, p'-Dichloro-diphenyltrichloroethane)	789-02-6	74	×	×	○	
pp'-DDT (p, p'-Dichloro-diphenyltrichloroethane)	50-29-3	74	×	○	○	
DDVP (2, 2-Dichloro-vinyldimethyl phosphate)	62-73-7	83	×	×		
		93				A○

Substance	CAS No.	Year	W	S	F	O
Decabromobiphenyl	13654-09-6	89	×	×	×	A×
Decabromodiphenyl ether	1163-19-5	77	×	×		
		87	×	○	×	
		88	×	○	×	
cis-Decahydro-naphthalene	91-17-8	84	×	×		
trans-Decahydro-naphthalene	191-17-8	84	×	○		
Decanol	112-30-1	79	×	×		
Diallylamine	124-02-7	81	×	×		
Diallyl phthalate	131-17-9	85	×	×		
1, 4-Diamino-anthraquinone	128-95-0	86	×	×		
4, 4'-Diamino-diphenylmethane	101-77-9	85	×	×		
1, 6-Diaminohexane	124-09-4	87	×	×		
1, 2-Diaminopropane	78-90-0	87	×	×		
1, 3-Diaminopropane	109-76-2	87	×	×		
2, 3-Diaminotoluene	2687-25-4	78	×	×		
2, 4-Diaminotoluene	95-80-7	78	×	×		
		90				A×
2, 6-Diaminotoluene	823-40-5	90				A×
o-Dianisidine	119-90-4	77	×	×		
Diazinon (O, O-Diethyl-O-2-isopropyl-4-methyl-6-pyrimidinyl phosphorothioate)	333-41-5	83	×	×		
		93				×
		93				A×
Dibenz [a, h] anthracene	53-70-3	89	○	○	○	A○
Dibenzofuran	132-64-9	83	×	×		
2, 2'-Dibenzothiazyl disulfide	120-78-5	77	×	×		
Dibenzothiophene	132-65-0	83	×	○		
p,p'-Dibenzoyl quinone dioxime	120-52-5	80	×			
Dibenzyl ether	103-50-4	84	○	○		
Dibenzyltoluene	26898-17-9	77	×	×		
o-Dibromobenzene	583-53-9	81	×	×		
m-Dibromobenzene	108-36-1	81	×	×		
p-Dibromobenzene	106-37-6	81	×	×		
1, 2-Dibromo-3-chloropropane	96-12-8	82	×	×		
		89	×	×		A×
Dibromocresyl glycidyl ether	22421-59-6	77	×	×		
1, 2-Dibromoethane	106-93-4	76	×	×	×	
		82	×	×		
		83				A○
1, 2-Dibromoethylene	540-49-8	81	×	×		
Dibromomethane	74-95-3	81	×	×		
Di-n-butylamine	111-92-2	86	×	×		

(*3) α-isomer(upper port) β-isomer (lower port)

Substance	CAS No.	Year	W	S	F	O
Dibutyldiglycol adipate	141-17-3	78	×	×		
2, 6-Di-t-butyl-4-ethylphenol	4130-42-1	84	×	○		
2, 5-Di-t-butylhydroquinone	88-58-4	80	×	×		
2, 6-Di-t-butyl-4-methylphenol (BHT)	128-37-0	76	×	○		
		77	×	○	○	
		85				A○
Di-n-butyl phthalate	84-74-2	74	○	○	○	R○
						P×
		75	○			
		82	○	○		
		85				A○
Dibutyl sebacate	109-43-3	81	×	×		
Dibutyltin compounds		83	×	○		
		84	×	○	×	
Dichloroacetic acid	79-43-6	84	×	×		
2, 3-Dichloroaniline	608-27-5	84	×	×		
2, 4-Dichloroaniline	554-00-7	76	○	○		
2, 5-Dichloroaniline	95-82-9	84	×	○		
2, 6-Dichloroaniline	608-31-1	84	×	×		
3, 4-Dichloroaniline	95-76-1	76	○	○		
		84	×	○		
3, 5-Dichloroaniline	626-43-7	84	×	×		
o-Dichlorobenzene	95-50-1	75	×	×	×	R×
		83				A○
m-Dichlorobenzene	541-73-1	75	×	○	×	R×
		83				A○
p-Dichlorobenzene	106-46-7	75	○	○	×	R×
		83				A○
3, 3'-Dichlorobenzidine	91-94-1	79	×	×		
Dichlorobromomethane	75-27-4	80				A○
		81	○	×		
		83				A○
3, 3'-Dichloro-4, 4'-diaminodiphenylmethane	101-14-4	79	×	×		
		85	×	×		
Dichlorodifluoromethane (Freon 12)	75-71-8	76				A○
		77				A○
1, 1-Dichloroethane	75-34-3	77	×	×		
		79				A×
		87	○	○		A○
		88	○	○		
1, 2-Dichloroethane	107-06-2	76	×	×	×	
		79				A○
		80				A○
		87	○	○		A○
		88	○	○		A○
1, 1-Dichloroethylene	75-35-4	79	×	×		

Substance	CAS No.	Year	W	S	F	O
cis-1, 2-Dichloro-ethylene (*4)	156-59-2	77	×	×		
		87	○	○		A○
trans-1, 2-Dichloro-ethylene(*4)	156-60-5	77	×	×		
		87	○	○		A○
Dichloromethane	75-09-2	79				A○
		80				A○
		83				A○
2, 3-Dichloro-1, 4-naphthoquinone	117-80-6	82	×	×		
2, 3-Dichloro-nitrobenzene	3209-22-1	81	×	×		
2, 4-Dichloro-nitrobenzene	611-06-3	81	×	×		
		94	×	×	×	A×
2, 5-Dichloro-nitrobenzene	89-61-2	81	×	×		
		94	×	×	×	A×
3, 4-Dichloro-nitrobenzene	99-54-7	81	×	×		
3, 5-Dichloro-nitrobenzene	618-62-2	81	×	×		
2, 3-Dichlorophenol	576-24-9	78	×	×		
2, 4-Dichlorophenol	120-83-2	78	×	×		
2, 5-Dichlorophenol	583-78-8	78	×	×		
2, 6-Dichlorophenol	87-65-0	78	×	×		
3, 4-Dichlorophenol	95-77-2	78	×	×		
3, 5-Dichlorophenol	591-35-5	78	×	×		
2, 4-Dichlorophenoxy-acetic acid	94-75-7	83	×	×		
1, 2-Dichloropropane	78-87-5	76	×	×	×	
1, 3-Dichloro-2-propanol	96-23-1	87	○	×	×	A×
2, 3-Dichloro-1-propanol	616-23-9	87	×	×	×	A×
1, 3-Dichloropropene	542-75-6	84	×	×		
2, 3-Dichloro-1-propene	78-88-6	88	×	×		A×
2, 2-Dichloropropionic acid	127-20-8	80	×	×		
		84	○	×		
2, 4-Dichlorotoluene	95-73-8	81	×	×		
2, 6-Dichlorotoluene	118-69-4	81	×	×		
3, 4-Dichlorotoluene	95-75-0	81	×	×		
Dicyclohexyl phthalate	84-61-7	85	×	×		
Dicyclopentadiene	77-73-6	78	×	○		
		89	×	×		
Dieldrin	60-57-1	74	×	×	×	
Diethanolamine	111-42-2	78	×			
Diethylamine	109-89-7	81	×	×		
N, N-Diethylaniline	91-66-7	77	×	×		
Diethylbiphenyl	28575-17-9	76	×	×	×	
Di (2-ethylhexyl) adipate	103-23-1	78	×	×		
		84				A○

(*4) The atmospheric sample of 1, 2-dichloroethylene is the total of *cis* and *trans* isomers.

I Monitoring Chemical Substances

Substance	CAS No.	Year	W	S	F	O
Di (2-ethylhexyl) phthalate	117-81-7	74	○	○	○	R○
						P○
		75	○			
		82	○	○		
		85				A○
Diethyl phthalate	84-66-2	85	×	×		
Di-n-heptyl phthalate	3648-21-3	82	○	○		
Diisobutylene	107-40-4	78	×	×		
Diisobutyl phthalate	84-69-5	74	○	○	○	R○
						P×
Diisodecyl adipate	6938-94-9	78	×	×		
Diisodecyl phthalate	26761-40-0	74	×	×	×	R×
						P×
Diisoheptyl phthalate	3648-21-3	74	○	○	○	R○
						P×
Diisopropylamine	108-18-9	81	×	×		
m-Diisopropylbenzene	99-62-7	77	×	×		
p-Diisopropylbenzene	100-18-5	77	×	×		
Diisopropyl fluorophosphate	55-91-4	93				A×
Diisopropylnaphthalene	38640-62-9	75	×	○	○	
		77	×	○	○	
		80	×	○	○	
Dilauryl phthalate	2432-90-8	85	×	×		
Dilaurylthiodipropionate	123-28-4	81	×	×		
Dimethoate (O, O-Dimethyl-S-methylcarbamoylmethyl dithiophosphate)	60-51-5	86	×	×		
		93	×	×	×	
4, 4'-Dimethoxy-diphenylamine	101-70-2	77	×	×		
Dimethylamine	124-40-3	86	×	○		
		91				A×
4-Dimethyl-aminoazobenzene	60-11-7	86	×	×		
2, 3-Dimetylaniline (2, 3-Xylidine)	87-59-2	76	×	○		
		90	×	×	×	A×
2, 4-Dimethylaniline	95-68-1	77	×	×		
2, 5-Dimethylaniline	95-78-3	76	×	○		
3, 4-Dimethylaniline	95-64-7	76	×	○		
		77	×	×		
3, 5-Dimethylaniline	108-69-0	76	○	○		
N, N-Dimethylaniline	121-69-7	76	○	○		
		90	×	○	×	A○
Di (α-methylbenzyl) phenol	2769-94-0	81	×	○		
Dimethyl-2, 2-dichloro-1, 2-dibromoethyl phosphate	300-76-5	84	×	×		
N, N-Dimethylformamide	68-12-2	78	×	×		
		91	○	○		A○
Substance	CAS No.	Year	W	S	F	O
1, 2-Dimethyl-naphthalene	573-98-8	84	○	○		
		85	×	○	○	
1, 3-Dimethyl-naphthalene	575-41-7 571-58-4 571-61-9	84	○	○		
		85	×	○	○	
1, 4-Dimethylnaphtha-lene; 1, 5-Dimethyl-naphthalene; 2, 3-Di-methylnaphthalene (*5)	581-40-8	84	○	○		
		85	×	○	○	
1, 8-Dimethyl-naphthalene	569-41-5	85	×	○	×	
2, 6-Dimethyl-naphthalene	581-42-0	84	○	○		
		85	×	○	○	
N, N-Dimethyl-p-nitrosoaniline	138-89-6	80	×			
2, 4-Dimethylphenol	105-67-9	82	×	×		
2, 5-Dimethylphenol	95-87-4	82	×	×		
3, 5-Dimethylphenol	108-68-9	82	×	○		
Dimethyl phthalate	131-11-3	85	×	×		
Dimethyl sulfoxide (DMSO)	67-68-5	92	○	○	○	
Dimethyl terephthalate	120-61-6	75	○			
		82	×	×		
2, 4-Dinitroaniline	97-02-9	90	×	○	×	
o-Dinitrobenzene	528-29-0	76	×	○	×	
		91	×	×		
m-Dinitrobenzene	99-65-0	76	×	○	×	
		91	×	×	×	
p-Dinitrobenzene	100-25-4	94	×	×	×	
2, 6-Dinitro-p-cresol	609-93-8	94	×	×	×	
4, 6-Dinitro-2-methylphenol	534-52-1	84	×	×		
1, 5-Dinitronaphthalene	605-71-0	85	×	×		
1, 8-Dinitronaphthalene	602-38-0	85	×	×		
2, 4-Dinitrophenol	51-28-5	84	×	×		
		94	×	×	×	
1, 3-Dinitropyrene	75321-20-9	90	×	×	×	
1, 6-Dinitropyrene	42397-64-8	90	×	×	×	
1, 8-Dinitropyrene	42397-65-9	90	×	×	×	A×
2, 4-Dinitrotoluene	121-14-2	76	×	×	×	
		91	×	×	×	
2, 6-Dinitrotoluene	606-20-2	76	○	○	×	
		91	×	×	×	
3, 4-Dinitrotoluene	610-39-9	76	×	×	×	
Di-n-octyl phthalate	117-84-0	74	○	○	×	R○
						P×
		82	×	×		
Dioctyl sebacate	122-62-3	81	×	×		

(*5) Figures indicate the total of the three types of dimethylnaphthalene

Substance	CAS No.	Year	W	S	F	O
Dioctyltin compounds		84	×	×		
Dioxane	123-91-1	76	×	×		
Dipentamethylene-thiuram tetrasulfide	120-54-7	80	×	×		
Diphenylamine	122-39-4	76	×	×	×	
		90	○	○	○	
Diphenyldisulfide	882-33-7	83	×	×		
Diphenylguanidine	102-06-7	78	×	×		
1, 1-Diphenylhydrazine	530-50-7	82	×	×		
N, N'-Diphenylhydrazine	122-66-7	86	×	×		
Diphenylmethane	101-81-5	83	×	○		
		84	○	○	○	
Diphenyltin compounds		89	○	○	○	
Distearyl thiodipropionate	693-36-7	81	×	×		
Dodecachlorododeca-hydrodimethanodibenzo-cyclooctene	13560-89-9	76	○	×	×	
Edifenphos (EDDP)	17109-49-8	93	×	×		
Endosulfan sulfate	1031-07-8	83	×	×		
Endrin	72-20-8	74	×	×	×	
Epichlorohydrin	106-89-8	77	×	×		
		86	×	×		
EPN	2104-64-5	86	×	×		
		93				A×
1, 2-Epoxy-3-phenoxy-propane	122-60-1	84	×	×		
2, 3-Epoxy-1-propanol	556-52-5	83	×	×		
Ethanolamine	141-43-5	80	×	×		
		94	○	○		A○
4-Ethoxyaniline (p-Phenetidine)	156-43-4	77	×	×		
		85	×	×		
6-Ethoxy-1, 2-dihydro-2, 2, 4-trimethylquinoline	91-53-2	80	×	×		
2-Ethoxyethanol	110-80-5	76	×	×		
2-Ethoxyethyl acetate	111-15-9	86	×	×		
Ethyl acrylate	140-88-5	80	×	×		
Ethylamine	75-04-7	81	×	×		
N-Ethylaniline	103-69-5	76	○	○		
		90	×	×	×	A○
2-Ethylanthraquinone	84-51-5	85	×	×		
Ethylbenzene	100-41-4	77	×	×		
		85	×	○		
		86	○	○	○	
Ethyl biphenyl	40529-66-6	76	×	×	×	
Ethyl bromide (Bromoethane)	74-96-4	76	×	×	×	
		83				A○
Ethyl chloride	75-00-3	77	×	×		
(Chloroethane)		79				A
		80				A○
		83				A○

Substance	CAS No.	Year	W	S	F	O
Ethyl p, p'-dichloro-benzilate	510-15-6	87	×	×	×	
Ethylene	74-85-1	77	○	○		
Ethylenechlorohydrin	107-07-3	80	×	×		
Ethylenediamine	107-15-3	87	×	○		
Ethylenediamine tetraacetic acid (EDTA)	60-00-4	79	×	○		
		94	○	×	×	
Ethylene glycol	107-21-1	77	×	×		
		86	○	×		
Ethylene oxide	75-21-1	80	×	×		
Ethyl formate	109-94-4	81	×	×		
2-Ethylhexanol	104-76-7	79	×	×		
2-Ethylhexyl acrylate	103-11-7	80	×	×		
Ethyl methacrylate	97-63-2	79	×	×		
N-Ethylmorpholine	100-74-3	79	×	×		
o-Ethylphenol	90-00-6	83	×	×		
m-Ethylphenol	620-17-7	83	×	×		
p-Ethylphenol	123-07-9	83	×	×		
Ethylthiometon	298-04-4	93				A×
Fenitrothion [O, O-Dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate]	122-14-5	83	×	×		
		93				A○
Fenthion (MPP)	55-38-9	93	×	×	×	A×
Fluorene	86-73-7	83	×	○		
		84	○	○	○	
Fluorobenzene	462-06-6	84	×	×		
Formaldehyde	50-00-0	75	×			
Fumaric acid	110-17-8	83	×	×		
Glycidyl methacrylate	106-91-2	86	×	×		
Glyoxal	107-22-2	80	○	○		
Glyphosate	1071-83-6	93	×	×	×	
α-HCH (α-Hexachloro-cyclohexane)	319-84-6	74	○	○	○	
β-HCH (β-Hexachloro-cyclohexane)	319-85-7	74	×	○	○	
γ-HCH (γ-Hexachloro-cyclohexane)	58-89-9	74	×	○	○	
δ-HCH (δ-Hexachloro-cyclohexane)	319-86-8	74	×	○	×	
Heptachlor	76-44-8	82	×	○	○	
		86				A×
Heptachloroepoxide	1024-57-3	82	×	○	○	
		86				A×
Heptanol	111-70-6	79	×	×		
Hexabromobenzene	87-82-1	77	×	×		
		81	×	○		

		82	×	○	×	
Hexabromobiphenyl	36355-01-8	89	×	×	×	A×
Hexabromocyclododecane	25637-99-4	87	×	○	○	

Substance	CAS No.	Year	W	S	F	O
Hexabromodiphenyl ether	36483-60-0	87	×	○	○	
		88	×	○	○	
Hexachlorobenzene (HCB)	118-74-1	74	×	×	○	
		75	×	○	○	
		78	○	○	○	
		94				A○
Hexachloro-1, 3-butadiene	87-68-3	81	×	×		
Hexachlorocyclo-pentadiene	77-47-4	81	×	×		
Hexachloroethane	67-72-1	76	×	×	×	
Hexachlorophene	70-30-4	81	×	○		
		82	×	○	×	
Hexamethyleneimine	111-49-9	86	×	×		
Hexamethylenetetramine	100-97-0	83	×	×		
Hexylene glycol	107-41-5	80	×	×		
4-(4-Hexylphenyl) benzonitrile	41122-70-7	85	×	×		
Hydrazine	302-01-2	86	×	×		
2-(2'-Hydroxy-3, 5'-di-t-butylphenyl)-5-chloro-benzotriazole	3864-99-1	80	×	×		
2-Hydroxy-3-naphtho-anilide (Azoic Coupling Component 2)	92-77-3	84	×	×		
2-Hydroxy-3-naphtho-(3-chloro-4, 6-dimethoxy) anilide (Azoic Coupling Component 12)	92-72-8	84	×	×		
2-Hydroxy-3-naphtho-(5-chloro-2-methoxy) anilide (Azoic Coupling Component 41)	137-52-0	84	×	×		
2-Hydroxy-3-naphtho-(4-chloro-2-methyl) anilide (Azoic Coupling Component 8)	92-76-2	84	×	×		
2-Hydroxy-3-naphtho-(3-nitro) anilide (Azoic Coupling Component 17)	135-65-9	84	×	×		
Iprobenfos (IBP)	26087-47-8	93	○	○	○	A×
Isobutyronitrile	78-82-0	77	×	×		
		87	×	×		A×
Isocyanuric acid	108-80-5	83	×	×		
Isophorone (3, 5, 5-Trimethyl-2-cyclohexene-1-one)	78-59-1	81	×	○		
Isophthalic acid	121-91-5	83	×	×		
Isophthalonitrile	626-17-5	77	×	×		
Isoprene	78-79-5	78	×	×		
Isopropanolamine	78-96-6	80	×	×		

Substance	CAS No.	Year	W	S	F	O
Isopropylamine	75-31-0	80	×	×		
		81	×	×		
Isopropylbenzene	98-82-8	77	×	×		
		85	×	○		
		86	○	○	○	
2-Isopropylnaphthalene	2027-17-0	84	×	○		
		85	×	○	○	
Isoprothiolane (Diisopropyl 1, 3-dithiolane-2-ylidenemalonate)	50512-35-1	92	○	○	○	A×
Isoxathion	18854-01-8	93				A×
Malathion	121-75-5	93	×	×	×	A×
Maleic acid	110-16-7	83	×	×		
Melamine	108-78-1	86	○	○		
		87	○	○	○	
		88			○	
		94	○	○	○	A○
2-Mercaptobenzimidazole	583-39-1	78	×	×		
2-Mercaptobenzothiazole	149-30-4	77	○	○		
		78	×	○	×	
2-Mercaptoimidazoline	96-45-7	83	×	×		
Methacrylic acid	79-41-4	87	×	×		
Methacrylonitrile	126-98-7	87	×	×		A×
Methidathion (DMTP)	950-37-8	93	×	×	×	A×
Methomyl {S-methyl-N-[(methylcarbamoyl) oxy] thioacetimidate}	16752-77-5	92	×	×	×	
Methoxybutanol	2517-43-3	80	×	×		
Methoxychlor [1, 1, 1-Trichloro-2, 2-bis (4-methoxyphenyl) ethane]	72-43-5	85	×	×		
Methoxybutyl acetate	4435-53-4	80	×	×		
2-Methoxyethanol	109-86-4	76	×	×		
2-Methoxyethyl acetate	110-49-6	86	×	×		
2-Methoxyphenol	90-05-1	86	×	○		
3-Methoxyphenol	150-19-6	86	×	×		
4-Methoxyphenol	150-76-5	86	×	×		
Methyl acrylate	96-33-3	80	×	×		
Methylamine	74-89-5	86	×	○		
N-Methylaniline	100-61-8	76	×	○		
		90	○	○	×	A○
α-Methylbenzylphenol	1988-89-2	78	×	×		
Methyl bromide	74-83-9	76	×	×	×	
		80				A○
Methyl chloride	74-87-3	79				A○

(Chloromethane)		80				A○
		83				A○
Methyl ethyl ketone	78-93-3	80	×	×		

Substance	CAS No.	Year	W	S	F	O
Methyl ethyl ketone oxime	96-29-7	78	×	×		
Methyl formate	107-31-3	81	×	×		
Methyl iodide	74-88-4	80				A○
Methylisobutylcarbinol	108-11-2	80	×	×		
Methyl isobutyl ketone	108-10-1	80	×	×		
Methyl mercaptan	74-93-1	92				A×
Methyl methacrylate	80-62-6	79	×	×		
1-Methylnaphthalene	90-12-0	76	×	×		
		84				A○
2-Methylnaphthalene	91-57-6	76	×	×		
		84				A○
2-Methyl-4-nitroaniline	99-52-5	85	×	×		
4-Methyl-2-nitroaniline	119-32-4	85	×	×		
4-Methyl-3-pentene-2-one	141-79-7	80	×	×		
2-Methylpiperidine	109-05-7	86	×	×		
2-Methylpyridine (α-Picoline)	109-06-8	86	×	○		
		87	○	○	○	
		94	○	○	○	A○
3-Methylpyridine (β-Picoline)	108-99-6	94	○	○	○	A○
4-Methylpyridine (γ-Picoline)	108-89-4	94	○	○	○	A○
3-Methylpyridine 4-Methylpyridine (*6)	108-99-6 108-89-4	86	×	○		
		87	○	○	○	
α-Methylstyrene	98-93-9	77	×	×		
β-Methylstyrene	5013-15-4	77	×	×		
m-Methylstyrene	100-80-1	77	×	×		
p-Methylstyrene	622-97-9	77	×	×		
MIPC (2-Isopropylphenyl-methylcarbamate)	114-26-1	88	×	×		A×
		94	×	×	×	
Molinate (S-Ethyl hexahydro-1H-azepine-1-carbothioate)	2212-67-1	92	○	○	×	A×
2-(Morpholinothio) benzothiazole	102-77-2	77	×	×		
Morpholine	110-91-8	79	×	×		
		94	○	○	×	A×
Mirex	2385-85-5	83	×	×		
MTMC (m-Tolyl methylcarbamate)	1129-41-5	88	×	×		A○
		94	×	×	×	
Naphthalene	91-20-3	76	×	×		
1-Naphthaleneacetic acid	86-87-3	84	×	×		
NAC (1-Naphthyl-N-methylcarbamate)	63-25-2	83	×	×		
		88	×	×		A×
1-Naphthol	90-15-3	77	×	×		
2-Naphthol	135-19-3	77	×	×		

Substance	CAS No.	Year	W	S	F	O
1, 4-Naphthoquinone	130-15-4	85	×	×		
1-Naphthylamine	134-32-7	76	×	○		
		79	×	○	×	
		85		×		
2-Naphthylamine	91-59-8	83	×	○		
		85		○		
Neopentyl glycol	126-30-7	77	×	×		
Nereistoxin	1631-58-9	93	×	×	×	
Nitrilotriacetic acid (NTA)	139-13-9	80	○	○		
		94	○	×	×	
3-Nitroacenaphthene	3807-77-0	84	×	×		
5-Nitroacenaphthene	602-87-9	84	×	×		
o-Nitroaniline	88-74-4	78	×	×		
		90	×	×	×	
m-Nitroaniline	99-09-2	78	×	×		
p-Nitroaniline	100-01-6	78	×	×		
		90	×	×	×	
o-Nitroanisole	91-23-6	76	○	○	×	
		91	×	○	○	
m-Nitroanisole	555-03-3	76	○	○	×	
p-Nitroanisole	100-17-4	76	×	×	○	
		91	×	×		
Nitrobenzene	98-95-3	76	○	○	○	
		77	○	○	○	
		86				A○
		91	○	○	○	A○
m-Nitrobenzoic acid	121-92-6	85	×	×		
5-Nitrobenzoimidazole	94-52-0	85	×	×		
Nitroethane	79-24-3	86	×	×		
Nitrofen (2, 4-Dichlorophenyl-4'-nitrophenyl ether)	1836-75-5	82	○	×		
3-Nitrofluoranthene	892-21-7	90	×	×	×	A○
Nitromethane	75-52-5	86	×	×		
2-Nitro-4-methylphenol	119-33-5	84	×	×		
3-Nitro-4-methylphenol	2042-14-0	84	×	×		
4-Nitro-3-methylphenol	2581-34-2	84	×	×		
5-Nitro-2-methylphenol	5428-54-6	84	×	×		
1-Nitronaphthalene	86-57-7	80	×	×		
o-Nitrophenol	88-75-5	78	×	×		
		79	×	×	×	
		94	×	×	○	A○
m-Nitrophenol	554-84-7	78	×	×		
		79	×	×	×	
		94	×	×	×	A×
p-Nitrophenol	100-02-7	78	○	×		
		79	×	×	×	

		94	×	×	×	A○

(*6) The values are the total of the two compounds.

Substance	CAS No.	Year	W	S	F	O
p-Nitrophenyl diethyl phosphate	311-45-5	93	×	×	×	
1-Nitropropane	108-03-2	79	×	×		
		86	×	×		
2-Nitropropane	79-46-9	79	×	×		
		86	×	×		
1-Nitropyrene	5522-43-0	90	×	×	×	A○
N-Nitrosodiethanolamine	116-54-7	94				A○
N-Nitrosodiethylamine	55-18-5	81	×	×		
N-Nitrosodimethylamine	62-75-9	81	×	×		
4-Nitrosodiphenylamine	156-10-5	77	×	×		
N-Nitrosodiphenylamine	86-30-6	90	○	×	○	
o-Nitrotoluene	88-72-2	76	○	○	×	
		86				A○
		91	×	×	×	A○
m-Nitrotoluene	99-08-1	76	○	○	×	
		86				A×
		91	×	×	×	
p-Nitrotoluene	99-99-0	76	○	○	×	
		86				A×
		91	○	×	×	
cis-Nonachlor	5103-73-1	82	×	○	○	
		86				A×
trans-Nonachlor	39765-80-5	82	×	○	○	
		86				A○
Nonanol	143-08-8	79	×	×		
Nonylphenol	25154-52-3	76	×	×		
		77	×	○		
Octabromodiphenyl ether	32536-52-0	87	×	○	×	
		88	×	○	×	
Octanol	111-87-5	79	×	×		
n-Octylamine	111-86-4	88	×	×		
p-Octylphenol	1806-26-4	77	×	○		
Octyltin compounds		84	×	×		
Organosilicone compounds		79	×	○		
		80	×	○	○	
Organotin compounds		75	×			
Oxamyl [Methyl N', N'-dimethyl-N (methylcarbamoyl) oxy-1-thio-oxamimidate]	23135-22-0	92	×	×	×	
Oxychlordane	26880-48-8	82	×	○	○	
		86				A×
PAP (O, O-Dimethyl-S-α-ethoxycarbonylbenzyl phosphorodithioate)	2597-03-7	88	×	×	×	A×
Pentabromobenzene	608-90-2	81	×	×		
Pentachloroaniline	527-20-8	81	×	×		
Pentachlorobenzene	608-93-5	75	×	×	○	R×
		79	×	○	○	
		94				A○
Pentachloroethane	76-01-7	84	×	×		
Pentachlorophenol	87-86-5	74	○	○		
PHC (o-Isopropoxyphenyl-methylcarbamate)	2631-40-5	88	×	×		A×
Phenanthrene	85-01-8	77	×	○		
Phenol	108-95-2	77	×	○		
Phenothiazine	92-84-2	86	×	×		
1-Phenyl-1-(2, 4-dimethylphenyl) ethane	6165-52-2	80	×	○	×	
1-Phenyl-1-(3, 4-dimethylphenyl) ethane	6196-95-8	75	×	○	×	
		77	×	○	○	
		80	×	○	×	
o-Phenylenediamine	95-54-5	78	×	×		
m-Phenylenediamine	108-45-2	78	×	×		
p-Phenylenediamine	106-50-3	78	×	×		
Phenylhydrazine	100-63-0	86	×	×		
N-Phenyl-1-naphthyl-amine	90-30-2	80	×	○		
		81	×	×	×	
N-Phenyl-2-naphthyl-amine	135-88-6	76	×	×	×	
		80	×	○		
		81	×	○	×	
o-Phenylphenol	90-43-7	78	×	×		
m-Phenylphenol	580-51-8	78	×	×		
p-Phenylphenol	92-69-3	78	×	×		
Phenyltin compounds		89	○	○	○	
Phorone (Diisopropylidene acetone)	504-20-1	81	×	×		
Phosalone	2310-17-0	93	×	×	×	A×
Phosmet (PMP)	732-11-6	93				A×
Phoxim (α-Cyanobenzyliden-amino O, O-diethyl phosphoro-thioate)	14816-18-3	88	×	×	×	A×
Total phthalates		75	○			
Phthalic acid	88-99-3	83	×	×		
o-Phthalonitrile	91-15-6	77	×	×		
Picric acid	88-89-1	80	×	×		
Piperazine	110-85-0	86	×	○		
Piperidine	110-89-4	86	×	×		
Piperophos	24151-93-7	93				A×
Polybrominated biphenyl (PBB)		81	×	×		
Polychlorinated naphtalene (PCN)		76	○	○	○	
		78	○	○	○	
Polychlorinated terphenyl (PCT)	6178-33-8	74	×	×	○	
		76	×	○	×	
		78	×	○	○	

Polyethyleneglycol aliphatic ester		82	×			

Substance	CAS No.	Year	W	S	F	O
Polyoxyethylene alkyl-amide		83	×	×		
Polyoxyethylene alkyl-amine		83	×	×		
Polyoxyethylene alkyl ether	27306-79-2	82	×	○		
Polyoxyethylene alkyl phenyl ether		77	○	○		
		78	○	○		
		82	○	○		
Polyoxyethylene-type nonionic surface active agents		82	○	○		
Probenazole (3-Allyloxy-1, 2-benzothiazole-1, 1-dioxide)	27605-76-1	92	×	×	×	
Propanil (N-3,4-Dichloro-phenyl propionamide)	709-98-8	80	×	×		
n-Propanolamine	156-87-6	80	×	×		
Propionaldehyde	123-38-6	87	×			A○
Propionitrile	107-12-0	87	×	×		A×
n-Propylamine	107-10-8	80	×	×		
Propylene	115-07-1	77	○	×		
Propylene glycol	57-55-6	77	×	×		
		86	○	○		
Propylene imine	75-55-8	86	×	×		
Propylene oxide	75-56-9	80	×	×		
Pyrene	129-00-0	89	○	○	○	A○
Pyridine	110-86-1	80	○	○		
		91	○	○	○	A○
Pyrrole	109-97-7	81	×	×		
Pyrrolidine	123-75-1	86	×	×		
Quinoline	91-22-5	84	○	○		
		91	×	○	×	
Quintozene (Pentachloro-nitrobenzene)	82-68-8	81	×	×		
		91	×	×	×	A○
Salithion (Dioxabenzofos)	3811-48-2	93				A×
Simazine [2-Chloro-4, 6-bis (ethylamino)-s-triazine]	122-34-9	80	×	×		
		91	×	×		
Simetryn [2,4-Bis (ethylamino)-6-methylthio-1, 3, 5-triazine]	1014-70-6	92	○	○	×	
Sodium alkylbenzene-sulfonate (branched chain)		77	×	×		
Sodium alkylbenzene-sulfonate (straight chain)		77	○	○		

Substance	CAS No.	Year	W	S	F	O
Sodium 4, 4'-bis (4-anilino-6-morpholino-1, 3, 5-triazine-2-yl) aminostilbene-2, 2'-disulfonate (Fluorescent-260)	16090-02-1	82	×	○		
Sodium 4, 4'-bis (2-sulfostyryl) biphenyl (Fluorescent-351)	27344-41-8	82	○	○		
Sodium m-nitrobenzene-sulfonate	127-68-4	77	×	×		
Sodium 4-[2-(5-nitro-2-furanyl) ethenyl] benzoate	54992-23-3	83	×	×		
Sodium salt of napthalenesulfonic acid-formalin condensate		79	×	×		
Solvent Yellow 14	842-07-9	88	×	×		
Styrene	100-42-5	77	×	×		
		85	×	○		
		86	○	○	○	
Telodrin	297-78-9	74	×	×	×	
Terephthalic acid	100-21-0	75	○			
		83	×	×		
o-Terphenyl	84-15-1	76	×	○	×	
		77	×	○	×	
m-Terphenyl	92-06-8	76	×	○	×	
		77	×	○	○	
p-Terphenyl	92-94-4	76	×	○	×	
		77	×	○	×	
1, 2, 4, 5-Tetrabromo-benzene	636-28-2	81	×	×		
Tetrabromobiphenyl	40088-45-7	89	×	×	×	A×
Tetrabromobisphenol A	79-94-7	77	×	×		
		87	○	○	×	
		88	×	○	×	
1, 1, 2, 2-Tetrabromo-ethane	79-27-6	76	×	×	×	
Tetrabromomethane	558-13-4	81	×	×		
1, 2, 3, 4-Tetrachloro-benzene	634-66-2	75	×	×	×	R×
1, 2, 3, 5-Tetrachloro-benzene	634-90-2	75	×	×	×	R×
1, 2, 4, 5-Tetrachloro-benzene	95-94-3	75	×	×	×	R×
2, 2', 3, 3'-Tetrachloro-4, 4'-diaminodiphenyl-methane	42240-73-3	85	×	×		
3, 3', 5, 5'-Tetrachloro-4, 4'-diaminodiphenyl-methane	25464-95-3	85	×	×		

Substance	CAS No.	Year	W	S	F	O
1, 1, 2, 2-Tetrachloro-ethane	79-34-5	76	×	×	×	
Tetrachloroethylene	127-18-4	74	○			R×
		75	○			R○
		79				A○
		80				A○
		83				A○
2, 3, 4, 6-Tetrachloro-phenol	58-90-2	78	×	×		
Tetraethoxysilane	78-10-4	92				A×
Tetraethylthiuram disulfide [Bis (diethyl-tiocarbamoyl) disulfide]	97-77-8	92	×			
2, 2, 3, 3-Tetrafluoropropionic acid	756-09-2	84	×	×		
Tetrahydrofuran	109-99-9	79	×	×		
Tetrahydronaphthalene	119-64-2	77	×	×		
Tetrahydrothiophene-1, 1-dioxide	126-33-0	76	×	×	×	
Tetramethylthiuram disulfide [Bis (dimethyl-thiocarbamoyl) disulfide]	137-26-8	85	×	×		
		92	×			
Tetramethylthiuram monosulfide [Bis (dimethylthiocarbamoyl) sulfide]	97-74-5	85	×	×		
		92	×	×		
Thiabendazole	148-79-8	86	×	×		
4, 4'-Thiobis (6-t-butyl-3-methylphenol)	96-69-5	81	×	×		
Thiophene	110-02-1	85	×	○		
Thiourea	62-56-6	77	×	×		
o-Tolidine	119-93-7	77	×	×		
Toluene	108-88-3	77	×	×		
		85	○	○		
		86	○	○	○	
o-Toluenesulfonamide	88-19-7	77	×	×		
		92	○	○		
p-Toluenesulfonamide	70-55-3	92	○	○		
p-Toluenesulfonyl chloride	98-59-9	77	×	×		
o-Toluidine	95-53-4	76	○	○		
		85				A×
m-Toluidine	108-44-1	76	○	○		
		85				A×
p-Toluidine	106-49-0	76	○	○		
		85				A×
p-Toluidine-2-sulfonic acid	88-44-8	80	×	×		
Toxophene	8001-35-2	83	×	×		
Triallylamine	102-70-5	81	×	×		
1, 3, 5-Tribromobenzene	626-39-1	81	×	×		
Tribromomethane (Bromoform)	75-25-2	76	×	×	×	
		80				A×
2, 4, 6-Tribromophenol	118-79-6	86	×	○		
2, 4, 6-Tribromophenyl (2-methyl-2, 3-dibromopropyl) ether	36065-30-2	79	×	×		
Tri-n-butylamine	102-82-9	86	×	×		
2, 4, 6-Tri-s-butylphenol	5892-47-7	84	×	×		
2, 4, 6-Tri-t-butylphenol	732-26-3	84	×	○		
Tributyl phosphate	126-73-8	75	○	○	○	
		77	○	○	○	
		93	○	○	○	A○
Tributyltin compounds		83	×	○		
		84	×	○	○	
Trichlorfon (DEP)	52-68-6	93	×	×	×	
Trichloroacetic acid	76-03-9	84	×	×		
2, 4, 5-Trichloroaniline	636-30-6	81	×	×		
2, 4, 6-Trichloroaniline	634-93-5	81	×	×		
1, 2, 3-Trichlorobenzene	87-61-6	75	×	×	×	R×
		79	○	○	×	
		86				A○
1, 2, 4-Trichlorobenzene	120-82-1	75	×	○	○	R×
		79	○	○	○	
		86				A○
1, 3, 5-Trichlorobenzene	108-70-3	75	×	×	×	R×
		79	○	○	○	
		86				A○
1, 1, 1-Trichloroethane	71-55-6	74	×			R×
		75	○			R×
		79				A○
		80				A○
		83				A○
1, 1, 2-Trichloroethane	79-00-5	76	×	×	×	
2, 2, 2-Trichloro-1, 1-ethanediol	302-17-0	86	×	×		
Trichloroethylene	79-01-6	74	○			R×
		75	○			R○
		79				A○
		80				A○
		83				A○
Trichlorofluoromethane (Freon 11)	75-69-4	76				A○
		77				A○
Trichloromethane (Chloroform)	67-66-3	74	○			R○
		75	○			R○
		79				A○
		80				A○
		83				A○

1, 1, 1-Trichloro-2-methyl-2-propanol	57-15-8	80	×	×		
		88	×	×		A○

Substance	CAS No.	Year	W	S	F	O
2, 4, 6-Trichloro-nitrobenzene	18708-70-8	84	×	×		
2, 4, 5-Trichlorophenol	95-95-4	78	×	×		
2, 4, 6-Trichlorophenol	88-06-2	78	×	○		
2, 4, 5-Trichlorophenoxy-acetic acid	93-76-5	83	×	×		
1, 2, 3-Trichloropropane	96-18-4	76	×	×	×	
1, 1, 2-Trichloro-1, 2, 2-trifluoromethane (Freon 113)	76-13-1	81	×	×		
		83				A○
Tricresyl phosphate (TCP)	1330-78-5	75	×	○	×	
		78	×	○	×	
		93	×	○	○	A○
Tricyclohexyltin compounds	13121-70-5	86	×	×		
Tridecyl alcohol	112-70-9	77	×	×		
Triethanolamine	102-71-6	78	×			
Triethylamine	121-44-8	81	×	×		
		91	○	○		
Triethylbiphenyl	42347-17-9	76	×	×	×	
Triethylene glycol ethyl ether	112-50-5	88	×	×		
Triethylene glycol methyl ether	112-35-6	88	×	×		
Triethyl phosphate	78-40-0	82	×	×		
Trifluralin	1582-09-8	94	×	×	×	
Trimellitic acid	528-44-9	86	×	×		
Trimethylamine	75-50-3	86	×	○		
		91				A○
1, 2, 3-Trimethylbenzene	526-73-8	76	×	×		
1, 2, 4-Trimethylbenzene	95-63-6	76	×	×		
1, 3, 5-Trimethylbenzene	108-67-8	76	×	×		
Tri (α-methylbenzyl) phenol	18254-13-2	81	×	○		
2, 2, 4-Trimethyl-1, 2-dihydroquinoline	147-47-7	80	×	×		
Trimethyl phosphate	512-56-1	82	×	×		
		84	×	×		
Trioctylamine	1116-76-3	81	×	×		
Trioctyl phosphate	1806-54-8	75	×	○	×	
Trioctyltin compounds		84	×	×		
Triphenyl hydride	61788-32-7	77	×	×		
Triphenylmethane	519-73-3	83	×	×		
Triphenyl phosphate	115-86-6	75	×	×	×	
Triphenyltin compounds		82	×	×		
		88	○	○	○	
Tripropyltin compounds		82	×	×		
Tris (2-bromoethyl) phosphate	27568-90-7	84	×	×		

Substance	CAS No.	Year	W	S	F	O
Tris (2-butoxyethyl) phosphate	78-51-3	75	×	○	×	
		78	×	×	×	
		93	○	○	○	A○
Tris (2-chloroethyl) phosphate	115-96-8	75	○	○	×	
		78	○	×	○	
		93	○	○	○	A○
Tris (2-chloroethyl) phosphite	140-08-9	84	×	×		
Tris (2-chloropropyl) phosphate	6145-73-9	84	×	×		
Tris (dibromopropyl) phosphate	126-72-7	75	×	×	×	
Tris (1, 3-dichloro-2-propyl) phosphate	13674-87-8	75	×	×	○	
		78	×	×	×	
		84	×	×		
Tris (2-ethylhexyl) phosphate	78-42-2	81	×	○		
1, 3, 5-Tris (2'-hydroxy-ethyl) isocyanurate	839-90-7	79	×	×		
Tris (2-hydroxypropyl) amine	122-20-3	81	×	×		
Tris (isopropylphenyl) phosphate	26967-76-0	78	×	○		
Trixylenyl phosphate	25155-23-1	81	×	○		
Vinyl bromide	593-60-2	81	×	×		
Vinyl chloride	75-01-4	75	○			
		79				A○
		80				A○
2-Vinylpyridine	100-69-6	91				A○
XMC (3, 5-Xylyl methylcarbamate)	2655-14-3	88	×	×		A×
o-Xylene	95-47-6	77	×	×		
		85	○	○		
		86	○	○	○	
m-Xylene	108-38-3	77	×	×		
		85	○	○		
		86	○	○	○	
p-Xylene	106-42-3	77	×	×		
		85	○	×		
		86	○	○	○	

I.4 Basic information and activities involved in environmental monitoring

(1) Toxicological information

Toxicological information is required to evaluate chemical effects on human health or other organisms in the environment. There are some databases available. The best organized, most comprehensives and most accessible is the World Health Organization's Environmental Health Criteria series. This series includes toxicological information on 160 chemicals.

(2) Preservation of environmental samples

From the chemical analytical standpoint, the main purpose of environmental sample preservation is to keep samples for further investigation in the future i.e. to re-check contaminant levels using new analytical methods, and to provide historical samples when new substances are to be analyzed. From the biological point of view, it is also important that samples are available for taxonomic analysis in the future. It is well known that these checks are only possible for samples which are filters passed through air samples, water samples, and biological samples. Such sample preservation is partly available in some conventional museums, but it is required to be ready for the long-term preservation system to freeze samples. Also registration of preserved samples is necessary in order to utilize samples for international monitoring.

(3) Understanding past environmental pollution - specimen banking

It is sometimes useful to understand historical trends in chemical environment pollution through analyzing preserved samples for pollution impacts. Historical pollution profiles and levels may be understood by chemical analysis of aged stratified materials, e.g. ocean or lake sediments, peat and glacier (ice) columns. Coral, annual rings in trees and other organisms, and historical museum collections can also provide further information. To extend this activity, it is fundamentally important to develop preservation methods for collected samples to enable their use as environmental records. It will then become possible to understand how pollution changes with the time by analyzing frozen or otherwise preserved soil, water, and biological samples, and even preserved air filters.

(4) Data Handling Systems

A wide variety of operations are involved in data handling, i.e. data accumulation, processing, transfer, publishing, storage, etc. Each operation has it own approach, for example whether each function are needed to be monitored. Therefore, data handling systems must include several measurement items and have to be designed with inherent flexibility to allow addition of historical data.

II Sampling

It is an absolute necessity that one attempts to collect samples that are representative of the matrix under investigation. When collecting samples, one must follow predetermined sampling protocols (procedures and methods) which have been chosen (bearing in mind the sampling (collection) site, the number of samples to be collected, and the timing of the sampling) to meet the purpose of the survey, and which are appropriate to the media being investigated.

II.1 Water quality

II.1.1 Sampling timing

Time the sampling trip such that it is possible to collect a representative water sample from the designated sampling point. Take into account factors such as the weather, tides, currents, geography etc.

II.1.2 Sampling point

For rivers, the primary sampling point is in the surface water layer (0-5 cm from the surface) at the centre of the main flow. However, the top 1-2 cm of this surface layer should be avoided so as not to collect floating dust, oil, etc. In addition, further samples can be collected through the full depth of the water column if required to meet the purpose of the study.

For lakes and the ocean, the sampling point will be selected after taking into consideration such factors as geography, whether there are freshwater (rivers or streams) or wastewater inflows, depth, tides, currents etc.

For underground water, the sampling site or sites will be selected after taking into consideration such factors as water flow and geological structure (hydrogeology), and also site conditions such as factories or land use, and avoiding bias so as to be able to understand the whole area's underground water.

II.1.3. Sampling tools and containers

Sampling tools

The type of water sampling tool required will depend on the sampling site and the type of sample to be taken. Sampling can be achieved using buckets, open water grab samplers (a ladle or bottle on the end of a long pole), or vertical and horizontal messenger activated samplers (such Niskin bottles or Kemmerer water samplers). The type of material such tools should be made of will depend on the purpose (target analytes) of the study, but relatively inert materials such as stainless steel, synthetic resin such as polypropylene, polyethylene or pertetrafluoroethylene (PTFE), or glass are all acceptable.

Sample containers

The size and type of sample to be taken will determine the type of sample container required.

- For volatile organic compounds, use clear or brown bottles or vials with screw caps or stoppers lined with tetrafluoroethene resin films, or similar products, which can be closed to provide a gas-tight seal.
- For semi-volatile or non-volatile organic compounds, use clear or brown glass jars with a stoppers or Teflon lined screw caps.
- For inorganic compounds such as heavy metals, use polyethylene or glass containers.

Cleanliness

Sampling tools and containers should be contamination free. The method and extent of cleaning will be determined by one's target analyte and predetermined instrumental detection limits. However, wherever possible use tools and containers which have been cleaned thoroughly. In particular,

- For volatile organic compounds, containers should be heated at 105 °C for 3 hours and then allowed to cool in a desiccator to avoid contamination immediately before use.
- For semi- and non-volatile organic compounds, use containers which have been washed with pesticide residue analysis grade solvent, and dried immediately before use.
- For heavy metal, use containers which have been washed with 10 % v/v nitric acid, or 16 % v/v hydrochloric acid, and then rinsed several times with pure (deionised) water.

II.1.4 Sampling operation

The number and volume (size) of the samples to be collected will depend on the number and concentration of the target analytes, the difficulty and expense of analysis, and whether extra storage is required. Collect the water sample using the most appropriate sampling tool given the nature of the sampling site, the target analyte, and the instrument on which quantitative measurement will be performed. Sample containers should be washed 3-4 times with water from the exact site of sampling prior to taking the sample. The samples should be carefully and gently poured into its container without making bubbles.

For underground water, collect spring water directly into sampling bottles when the groundwater is gushing out. Artesian wells are sampled by using water samplers avoiding mixing in floating objects. For pump well, collect water samples directly with sampling bottles after first totally exchanging the water contained in the pump.

- For volatile organic compounds, sample containers should be completely filled with bubble-free water and sealed tight.
- For semi- and non-volatile organic compounds, sample container should be completely filled with water that is as free as possible of air bubbles.
- For inorganic compounds such as heavy metals, the container should be approximately 80 - 90 % filled with the water sample (the space above the surface of the water sample allows thorough mixing just prior to analysis.

II.1.5 Field records

On a form which has been prepared in advance, record all pertinent details e.g. the sampling date, sample name (code), sampling site's name (code), an accurate position for the sampling site (map of G.I.S. position), general environmental conditions such as the nature of the surrounding landscape, the state of the tide or river flow, weather conditions such as cloud cover and air temperature, and general water conditions such as colour, water temperature, pH, and dissolved oxygen content, etc.

II. 1.6 Labelling of samples

Label each sample unambiguously, i.e. write on the sample bottle in water-resistant ink details of the name or code of the sample, the sampling date, the sampling site name etc. Alternatively, one may attach a label to the sample bottle detailing the same information.

Remember also to record these details in the field record mentioned earlier. This provides a back-up record of the sampling sequence and allows cross-checking of the analytical results with field data, and reduces future problems with identification (confusion) of stored samples. Finally, record on each sample and in the field record details of transport methods, storage methods, etc.

II.1.7 Transport and storage of samples

Procedures for handling the sample during transport will depend on the nature of the sample matrix and the target analytes. However, ideally all samples should be cooled in ice soon after collection, and then transported to the laboratory packed in ice. If samples must be stored for any significant period of time, refrigerate or freeze samples as soon as possible after collection or arrival at the laboratory.

II.2 Sediment

II.2.1 Sampling timing

If only sediment is to be sampled, time the sampling trip such that it is possible to collect a representative sample from the designated sampling point taking into account factors such as the weather, tides, currents, geography etc. If sediment sampling is linked to a study of the overlying water, then collect the sediment sample at the same time as the water samples.

II.2.2 Sampling point

In rivers, the sampling point will be (a) the point where water samples are taken if the sediment study is linked to a water quality study, or (b) if only sediment is being studied, that part of the river where sedimentation is occurring (the places in the river where sediment is being deposited such as the outside of bends).

For lakes and the ocean, the sampling point will be selected after taking into consideration such factors as geography, whether there are freshwater (rivers or streams) or wastewater inflows, depth, tides, currents etc.

II.2.3 Sampling tools and containers

Sampling tools

The type of sediment sampling tool required will depend on the sampling site, the nature of the sediment, and the type of sample to be taken. Sampling can be achieved using gravity, hand or messenger activated core samplers, or for larger samples use grab dredges (such as Peterson or Ekman dredges).

Sample containers

The size and type of sample, the nature of the target analytes and their detection limits will determine the type of sample container required.

- For volatile organic compounds, use clear or brown bottles or vials with Teflon lined screw caps or stoppers which can be closed to provide a gas-tight seal.
- For semi-volatile or non-volatile organic compounds, use clear or brown glass jars with a stoppers or Teflon lined screw caps.
- For inorganic compounds such as heavy metals, use polyethylene or glass containers.

Cleanliness

Sampling tools and containers should be contamination free. The method and extent of cleaning will be determined by one's target analyte and predetermined instrumental detection limits. However, wherever possible use containers which have been cleaned thoroughly.

- For volatile organic compounds, containers should be heated at 105 °C for 3 hours and then allowed to cool in a desiccator to avoid contamination immediately before use.
- For semi- and non-volatile organic compounds, use containers which have been washed with pesticide residue analysis grade solvent, and dried immediately before use.
- For heavy metal, use containers which have been washed with 10 % v/v nitric acid, or 16 % v/v hydrochloric acid, and then rinsed several times with pure (deionised) water.

II.2.4 Sampling operation

The number and volume (size) of the samples to be collected will depend on the number and concentration of the target analytes, the difficulty and expense of analysis, and whether extra storage is required. Collect the sediment sample using the most appropriate sampling tool given the nature of the sampling site, the target analyte, and the instrument on which quantitative measurement will be performed. In rivers, take the sediment sample from three equidistant

positions within a 50 m diameter circle. Pool (mix) the samples. Cylindrical samples may be taken depending on the survey's purpose. Take cylinders of mud about 10 cm deep every meter.

In lakes and in the ocean, take sediment (mud) samples from three equidistant grid positions. The number of sampling points may be increased if there are special circumstances, such as the inflow of fresh or waste waters nearby. Samples may be taken through the full depth of the sediment should the survey require such sampling.

- For volatile organic compounds, take samples into the sealed container without gap and seal up.
- For semi-volatile and non-volatile organic compounds, place the sample on a clean, stainless steel tray, remove large impurities such as stones, shells, flakes of animals and plants, thoroughly mix the remaining sample, and place the sample in its container.
- For heavy metals, use a clean polyethylene, polypropylene or PTFE tray, remove large impurities, mix the remaining sample, and place the sample in its container.

II.2.5 Field records

On a pre-prepared form, record such details as the sampling date, sample name (code), sampling site's name (code), an accurate position for the sampling site (map of G.I.S. position), general environmental conditions such as the nature of the surrounding landscape, the state of the tide or river flow, weather conditions such as cloud cover and air temperature, and general water conditions such as water temperature, pH, dissolved oxygen content, and colour (visually) etc. In addition, record information such as sediment temperature, appearance and colour, odour, any obvious animal or inanimate particulate matter, and mud rate.

II.2.6 Labelling of samples

Label each sample unambiguously, i.e. write on the sample bottle in water-resistant ink or attach a label to the sample bottle detailing the name or code of the sample, the sampling date, the sampling site name etc.

II.2.7 Transport and storage of samples

Procedures for handling the sample during transport will depend on the nature of the sample matrix and the target analytes. When samples arrive at laboratory,

- Samples for volatile organic compound analysis should be weighed and analysed as soon as possible.
- Samples for semi-volatile and non-volatile organic compound analysis should be screened with a 1 mm mesh sieve (16 mesh), or centrifuged to remove larger particles, and thoroughly remixed prior to analysis. If samples must be stored, refrigerate or freeze samples after sieving.
- Samples for heavy metal analysis are sorted with non-metal sieve after air dry, thoroughly mixed and analysed.

In all cases, the mud rate (the ratio of sample weight passing through the sieve : total sample weight prior to sieving) should be calculated. And the interstitial moisture content should be determined by drying part of the samples in the oven (105-110 °C, about 2 hours). Finally, the total organic content of the sediment should be calculated by heating (ashing) the sample in the oven (600 ± 25 °C, about 2 hours).

II.3 Soil

II.3.1 Sampling timing

There are fewer restrictions on when soil samples may be collected. However, one should still time the sampling trip so that it is possible to collect a representative soil sample from the designated sampling point. Take into account factors such as the weather, season, geography etc.

II.3.2 Sampling point

The sampling point will be selected after taking into consideration such factors as local geography, soil vegetation coverage, whether there are freshwater or wastewater channels or subsurface drains, etc.

II.3.3 Sampling tools

Sampling tools

The type of water sampling tool required will depend on the sampling site and the type of sample to be taken. Sampling can be achieved using trowels, spades, or augers. Such tools are normally made of stainless steel.

Sample containers

The size and type of sample to be taken will determine the type of sample container required.

- For volatile organic compounds, use clear or brown bottles or vials with Teflon lined screw caps or stoppers which can be closed to provide a gas-tight seal.
- For semi-volatile or non-volatile organic compounds, use clear or brown glass jars with a stoppers or Teflon lined screw caps.
- For inorganic compounds such as heavy metals, use polyethylene or glass containers.

Cleanliness

Sampling tools and containers should be contamination free. The method and extent of cleaning will be determined by one's target analyte and predetermined instrumental detection limits. However, wherever possible use tools and containers which have been cleaned thoroughly. In particular,

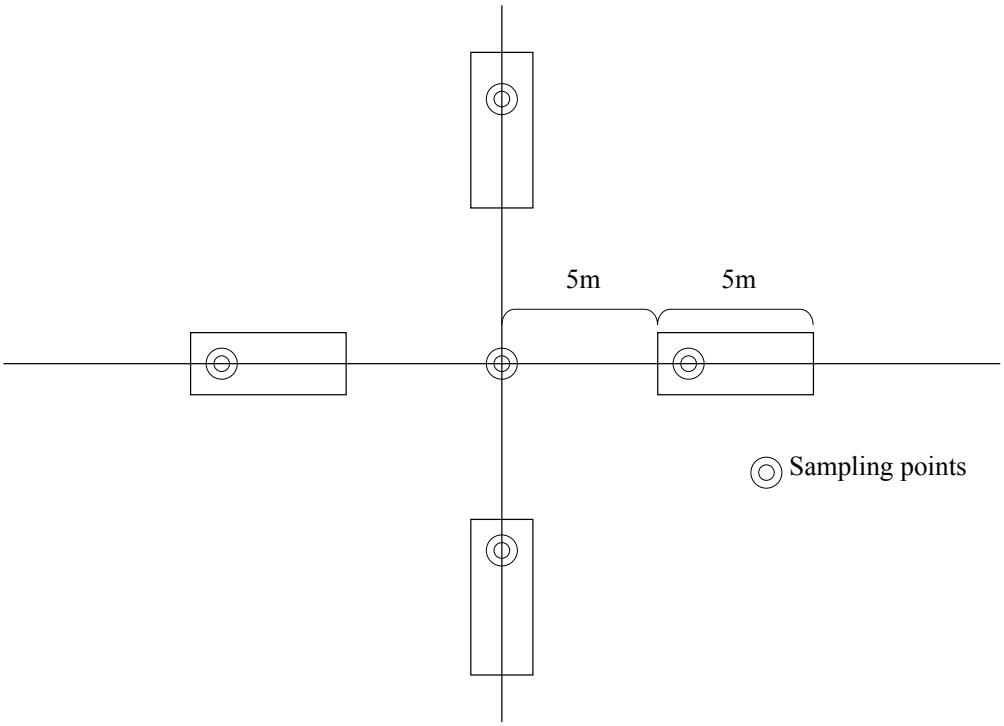
- For volatile organic compounds, containers should be heated at 105 °C for 3 hours and then allowed to cool in a desiccator to avoid contamination immediately before use.
- For semi- and non-volatile organic compounds, use containers which have been washed with pesticide residue analysis grade solvent, and dried immediately before use.
- For heavy metal, use containers which have been washed with 10 % v/v nitric acid, or 16 % v/v hydrochloric acid, and then rinsed several times with pure (deionised) water.

II.3.4 Sampling operation

The number and volume (size) of the samples to be collected will depend on the number and concentration of the target analytes, the difficulty and expense of analysis, and whether extra storage is required. Take sufficient, appropriately spaced surface soil samples (top 5 cm of the soil profile) using a geographical map to meet the survey's requirements. For each sampling point, take 3-5 samples from the same area, and mix them well to form one pooled sample. Take sub-surface

soil and soil column (profile) samples where required by the survey using an auger. Since soil and ground water pollution are closely related, combine soil and groundwater surveys where possible.

A typical sampling method for dioxin analysis (**Figure II-3-1**) is shown for reference material.



Note : Sampling is conducted by the 5 points mixture method from a 10 m square plot of bare land. Make samples by mixing 5 samples ; one taken from the centre of the plot, and the others from points between 5 to 10 m in each of four directions.

Figure II-3-1 Reference example of 5 sampling points method
(cited from "Provisional manual for soil survey related to dioxins", Soil and Pesticide Division, Water Quality Reservation Bureau, Japan Environment Agency, Jan. 1998)

II.3.5 Field records

On a form which has been prepared in advance, record such details as sampling date, sample site, an accurate map position, height above sea level, sampling location at the site, soil depth, sample's name or code, general environmental conditions, any obvious pollution sources in the surrounding locality, land use patterns, geographical features, and weather conditions. In addition, record such details as vegetative coverage at the site, drainage patterns, local geology, and soil characteristics such as colour, any obvious impurity, muddiness or dryness etc.

II.3.6 Labelling of samples

Label each sample unambiguously, i.e. write on the sample container or attach a label detailing the name or code of the sample, the sampling date, the sampling site name etc.

II.3.7 Transport and storage of samples

Procedures for handling the sample during transport will depend on the nature of the sample matrix and the target analytes. However, ideally all samples should be cooled in ice soon after collection, and then transported to the laboratory packed in ice. If samples must be stored for any significant period of time, refrigerate or freeze samples as soon as possible after collection or arrival at the laboratory.

II.4 Air

II.4.1 Sampling timing

Timing of the sampling will to a large extent depend on the purpose of the survey. However, endeavour to ensure that as representative an air sample as possible is collected i.e. sample only during stable climatic conditions. The time period over which the sample is collected will depend on the concentration and stability of the target chemicals.

II.4.2 Sampling point

Choose a sampling point where the local atmosphere conditions are well understood, and avoid places which are directly influenced from specific fixed or mobile pollution sources. Place the air intake of the sampling device at least 1 m away from any floor or wall surface.

II.4.3 Sampling tools and methods

Use either a high volume air sampler or low volume air sampler within a shelter. Trap particles in the air using filters made of quartz fibre, fluorine resin, or nitro-cellulose. Measure air flow at both the start and end of sampling, and calculate the amount of absorbed air. In addition, weigh the filters before and after sampling. Weigh the filters at 20 °C and a relative humidity of 50%. Calculate the sample weight and concentration of medium size particle substances in the air.

Choose a sample collection system based on the chemical nature of the target analyte, its concentration, and the nature of the sampling site. Suck the air sample into either stainless steel containers inactivated by electrolytic polishing or coated by oxidation or with silica, glass vacuum bottles, elasticised gas sampling bags (10 - 30 L), single or multiple use cartridges filled with absorbent polymer beads such as Tenax GC, Chromosorb, XAD, carbon molecular sieve or activated charcoal, or gas washing bottles filled with freshly prepared absorbent liquid. Suck the air through cartridges or the liquid in gas washing bottles at a flow rate optimised to trap the greatest amount of sample without break-through or contamination.

Wash sample containers, cartridges or gas washing bottles well prior to sampling. Use only equipment which has been shown not to contaminate the sample above predetermined instrumental detection limit. Use piping made of materials which has been shown not to contain or absorb the target chemicals, such as glass lined stainless steel, glass lined aluminium or aluminium with an oxidised coating, fluorine resin, or polyimide. Use a pump to pressurise the system and check for air leakage before use.

Figure II-4-1～II-4-4 shows a typical sampling apparatus. If possible, place all of such apparatus indoors, except for the sampling pipe. If it is not possible to set up the apparatus indoors, set up apparatus on a sturdy tripod, or use weights to prevent the apparatus falling over, and cover the trap and tubing or sampling bottle with aluminium foil etc.

Bearing in mind the chemical nature of the target chemicals and the atmospheric conditions at the air intake, use a dehumidifier which doesn't absorb the target substances at high percentage humidity, and use filter materials which traps dust but not the target substance. Finally, use an umbrella or other such glass or stainless steel funnel shaped cover to avoid rain ingress.

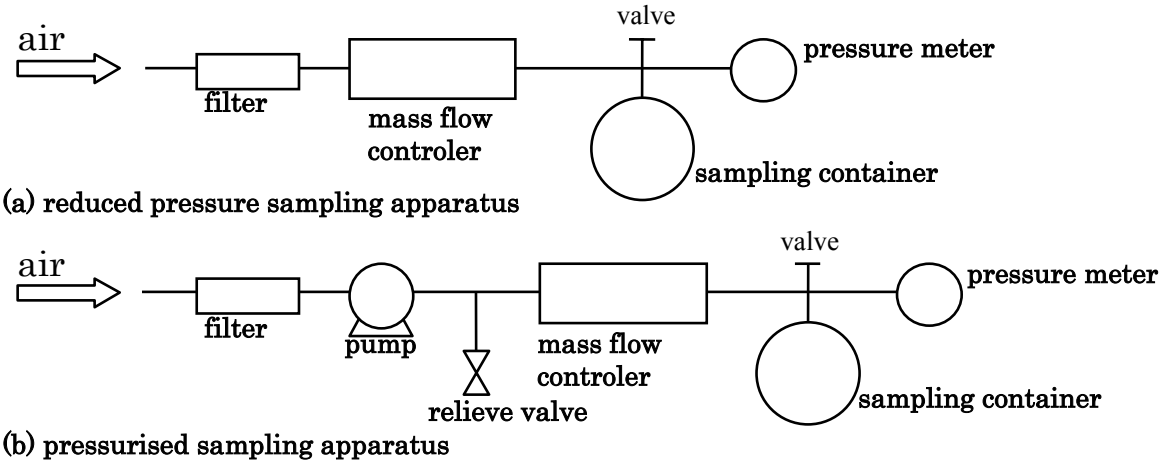


Figure II-4-1 Outline of sampling apparatus by container sampling method

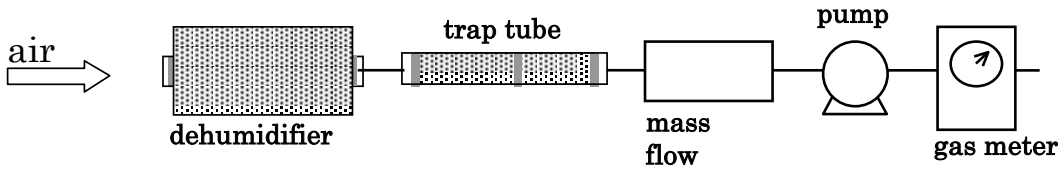


Figure II-4-2 Outline of sampling apparatus by solid phase adsorption - dissolution extraction method

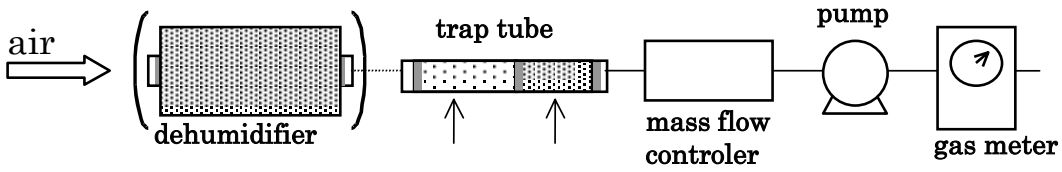


Figure II-4-3 Outline of sampling apparatus by solid phase adsorption-thermal desorption method *

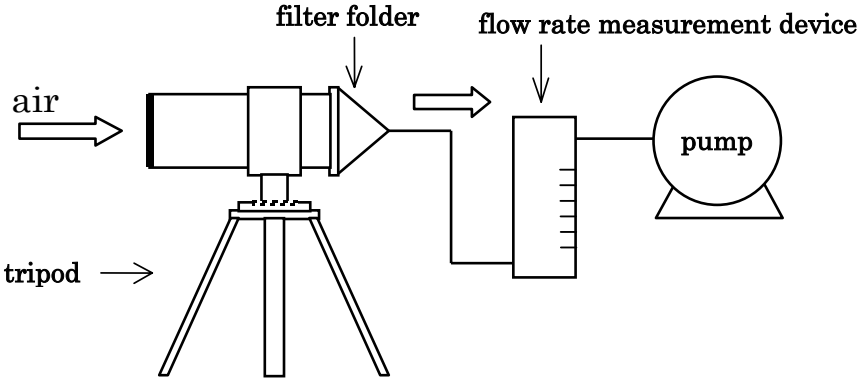


Figure II-4-4 Low volume air sampler *

* cited from "Manual of analytical method for air pollution" Japan Environment Agency, Feb1997

II.4.4 Record of field research

On a form which has been prepared in advance, record all pertinent details e.g. sampling date, sample name (code), sampling site's name (code), an accurate position for the sampling site (map of G.I.S. position), general environmental conditions such as the nature of the surrounding landscape. In addition, record climatic conditions such as temperature, humidity, atmospheric pressure, wind direction, wind velocity, the sampling method, the start time and finish time of sampling, sample temperature, pressure, sampling air flow rate, accumulated air flow amount at gas meter etc.

II.4.5 Labelling of samples

Label each sample unambiguously, i.e. write on the sample bottle in water-resistant ink, or attach a label with details of the name or code of the sample, the sampling date, the sampling site name etc.

II.4.6 How to transfer and store samples

Construct and take apart apparatus quickly to avoid contamination from surrounding air. Put filters in clean plastic bags. Cover glass vacuum bottles and gas sampling bags with black bags to prevent deformation by the sun. Close sampling containers tightly, or pressurise depending on sampling method to avoid outside influence. Seal cartridges and place in resealable containers with activated charcoal etc. In all cases, transfer the sample containers quickly after sampling and analyse as soon as possible. If it is impossible to analyse within a short period of time, store them in a dark room and avoid outside air. Analyse as soon as practicable.

II.5 Living things

II.5.1 Capture

Capture organisms at the same time every year, taking into account their breeding cycles and migration patterns. Standardise the time, place, number and size of organisms captured in order to maintain high precision. In general, choose juveniles since they are most sensitive to environmental pollution.

II.5.2 Capture method

II.5.2.1 Fish

The method of capture will depend on the type of fish to be caught e.g. one can use hand nets, drop nets, fish-traps, gill nets, seine nets, drift nets, drag nets, hook and line, or electrofishers. Whatever method is used, use equipment and sample containers which are not contaminated with the target substances to avoid sample contamination. Measure the length of each fish caught, and determine its weight. Place each sample in clean, non-contaminated artificial resin bags, or hard quality glass containers.

II.5.2.2 Shelled organisms

The method of capture will depend on the type of shelled organism. Collect shelled organisms e.g. snails, mussels, abalone etc., by hand or dredge from the substrate upon which they live i.e. plants, rocks or the river, lake or ocean floor. After collecting the organism, place the samples into clean, non-contaminated artificial resin bags, or hard quality glass containers.

II.5.2.3 Birds

The method of capture will depend on the type of bird to be collected and its habits. Capture birds by hand, net, trap or gun etc. Determine the bird's body weight and measure the length of the longest part of the wing. Put each bird in its own clean, non-contaminated artificial resin bags, or hard quality glass containers.

II.5.3 Record of field research

On a form which has been prepared in advance, record all pertinent details e.g. sampling date, sample name (code), sampling site's name (code), an accurate position for the sampling site (map of G.I.S. position), general environmental conditions such as the nature of the surrounding landscape. In addition, record the species name, the organism's body weight, length, capture number, and the total number of organisms caught, and the capture method.

II.5.4 Transfer and storage

Sacrifice (kill) the organisms at the site of capture in the appropriate, ethical manner. Freeze biological organisms on site, or chill in ice during transfer. Prepare samples and analyse them as soon as possible after arrival at the laboratory. If the samples are required to be stored, put the

samples into bags or containers which do not contain, dissolve or absorb the target substances. Weigh the samples before and after sample preparation, and freeze or refrigerate them in order to avoid contamination or decomposition.

II.5.5 Sample labelling

Label each sample unambiguously, i.e. write on the sample bottle in water-resistant ink, or attach a label with details of the name or code of the sample, the sampling date, the sampling site name etc.

II.6 Wastes

II.6.1 Sampling timing

Time the sampling trip such that it is possible to collect a representative wastewater sample from the designated sampling point. Sample at the start and finish of waste treatment, or at regular intervals, or when there are suspicions about leakage.

II.6.2 Sampling point

The place of sampling will depend on the nature of the wastewater survey, the nature of the sampling site and the type of sample to be taken. Take samples of exudate water, groundwater, overflowing water (surface run off), target area, surrounding air, leakage from intermediate treatment sites, last treatment place, landfill sites, waste disposal sites, areas which are considered likely to leak or where there is a significant risk of contamination during collection, transport, and storage of wastes.

II.6.3 Sampling tools, sampling operation, transfer and storage of samples

The type of water sampling tool required will depend on the sampling site and the type of sample to be taken. In general, sampling tools acceptable for water, air or soil sampling will be acceptable for sampling wastes. When sampling waste of homogeneous character, take samples that are consistent in character and composition of the bulk of the wastes. When sampling waste of inhomogeneous character, take multiple samples of the waste, and mix in order to gain

representative samples. In either case, crush and homogenise the final pooled sample, and then prepare the samples for analysis.

II.6.4 Record of filed research

On a form which has been prepared in advance, record all pertinent details e.g. the sampling date, sample name (code), sampling site's name (code), an accurate position for the sampling site (map of G.I.S. position), general environmental conditions such as the nature of the surrounding landscape. In addition, record details such as the purpose of sampling, the condition and treatment of wastes, the causes of any accidents, the kind of wastes, etc.

II.6.5 Display and coding of samples

Label each sample unambiguously, i.e. write on the sample bottle in water-resistant ink, or attach a label with details of the name or code of the sample, the sampling date, the sampling site name etc.

II.7 Sampling flow chart

An example of a sampling flow chart for the analysis of semi- and non-volatile compounds in river water is shown in **Figure II-7-1**.

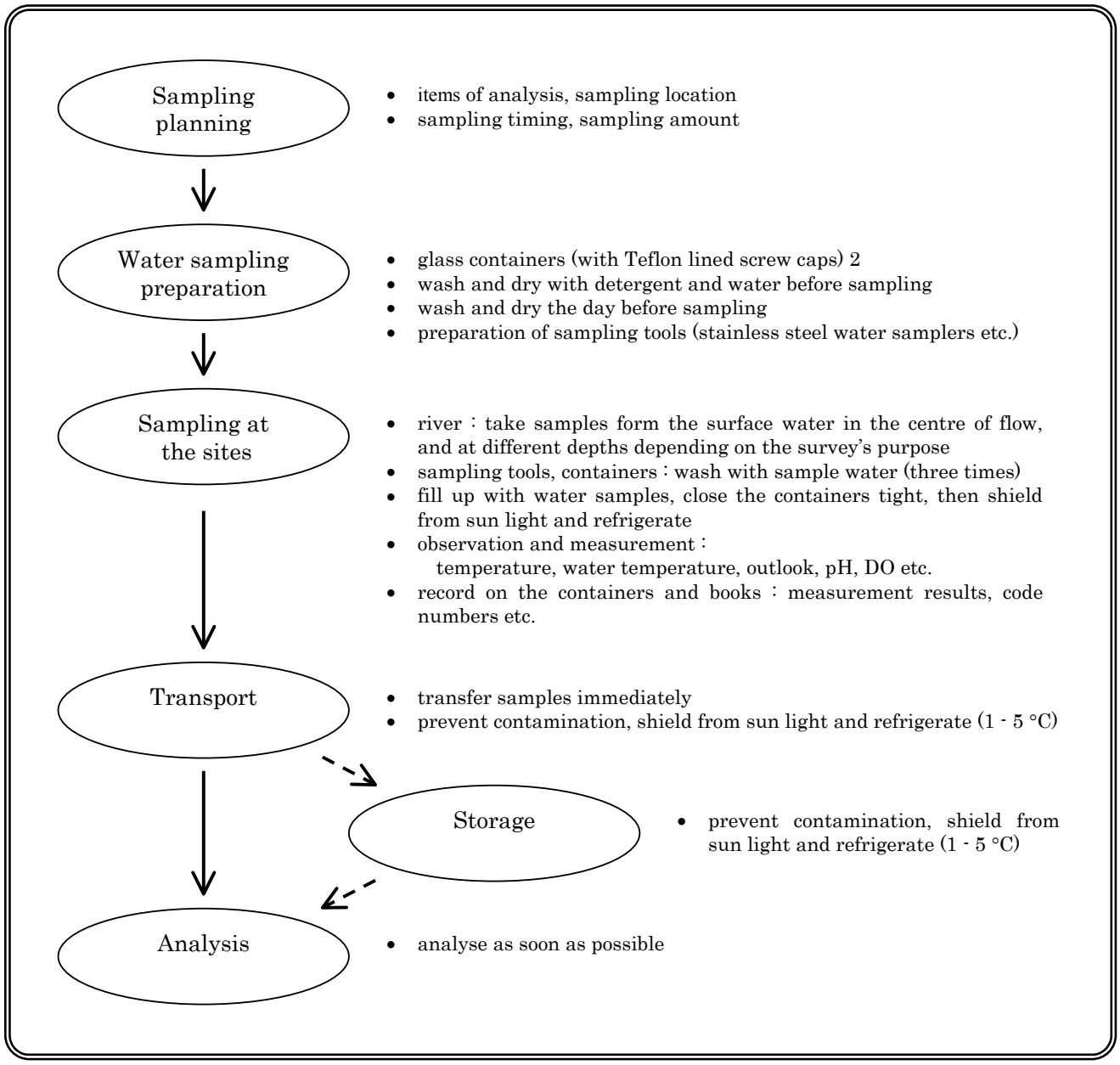


Figure II-7-1 An example of a sampling flow chart for analysis of semi- and non-volatile compounds in river water

III Analytical Methods

III.1 Outline of chemical analysis in the environment

III.1.1 Analytical order of chemicals in the environmental samples

In order to be able to determine very small amounts (very low concentrations) of chemicals in the environment, it is necessary to follow a series of operations :

1) isolation (extraction and separation) of the target chemicals from sample matrix (air, water, sediment, living things, etc.).
2) separation and purification of the target chemical from co-extracted, non-target chemicals (sample clean-up).
3) sample concentration.
4) measurement by highly selective and sensitive analytical equipment, such as gas chromatography / mass spectrometry (GC/MS). Occasionally it is also necessary to derivatise (chemically modify) non-volatile and heat degradable target chemicals prior to analysis by the two most commonly used instrumental methods in environmental analysis, GC or GC/MS.

The following sections describe this series of analytical operations in more detail, and introduce some of the main analytical methods for environmental pollutants.

III.1.2 Sample preparation methods

III.1.2.1 Extraction / separation methods

A. Water samples

a) Selection of extraction methods

It is possible to categorise chemical substances and their solubility in water based on simple physico-chemical characteristics, such as their molecular weight, boiling point, and polarity (**Figure III-1-1**). Methods for isolating chemicals from water based on these broad chemical categories are shown in **Figure III-1-2**.

volatility →

polarity ↑	volatile	semi-volatile	non-volatile
polar	alcohols ketones carboxylic acids	alcohols ketones carboxylic acids phenols	high molecular electrolytes carbohydrates fulvic acid
semi-polar	ethers esters aldehydes	ethers esters aldehydes epoxides heterocyclic compounds	protein carbohydrates humic acids
non-polar	aliphatic compounds aromatic compounds	aliphatic compounds aromatic compounds alicyclic compounds	non-ionic polymers lignin
	small	medium	large

molecular weight →

Figure III-1-1 Classification of chemicals by physicochemical character[1)]

volatility →

polarity ↑	volatile	semi-volatile	non-volatile
polar	none liquid-solid phase extraction distillation	derivatisation liquid-liquid extraction liquid-solid extraction pH adjustment	reduced pressure distillation freeze dry
semi-polar	purge & trap	liquid-liquid extraction liquid-solid extraction	reversed permeation
non-polar	head space	liquid-liquid extraction liquid-solid extraction	ultrafiliration
	small	medium	large

molecular weight →

Figure III-1-2 Chemical separation method for water samples[1)]

b) Volatile organic compounds

The two main methods for the isolation of volatile organic compounds from water are the purge and trap method and headspace method. These are described below;

(1) Purge and Trap Method (P&T)

This method, also known as the *dynamic headspace method*, removes (separates) volatile compounds from the sample matrix (in this case, water) by passing an inert gas such as helium or nitrogen through the matrix (purging). The target, volatile compounds are desorbed from the aqueous phase to the gas phase (purged) and are then separated from the stream of gas (trapped) by adsorbent filters. The adsorbent material is then heated in a stream of GC carrier gas (usually pure helium). This releases the trapped substances into the carrier gas, the target analytes are introduced to GC, and analysed. Typical trapping (adsorbent) materials are porous polymer beads, activated charcoal, silica gel, other GC column packing materials, or combinations of such materials.

Practically, this method can only be undertaken using one of a few specified instruments which are fully automated from the purging step, through trapping, releasing the target analytes, and introduction into to the GC.

The P&T method has following features and points:

1) The method is appropriate for micro-level analysis (ppt - ppb concentrations) because it is possible to analyse the whole volatile compounds in water samples.
2) The gas used to purge the sample absorbs significant amounts of water in addition to the volatile chemicals. One must remove this water prior to analysis because water causes interference in the GC/MS system.
3) After the volatile chemicals are released from the adsorbant by heating, one must introduce these compounds into GC column as a narrow band. If wide bore column is used, there is no need for any special treatment because it is possible to pass large carrier gas flows into the GC/MS system. However, when regular bore column is used, the end of column must be cooled (cryo-focused) at -100°C by liquid nitrogen to narrow the width of the band of chemicals.
4) Some volatile compounds are found extensively in the environment. The P&T method is highly sensitive, and analysis tends to be influenced by such extraneous, volatile compounds found in the purge gas and surrounding atmosphere. It is therefore necessary to protect the purified purge gas and the analytical system from contamination. Blank samples should be treated in the same manner as other sample in order to make sure there is no

contamination.

(2) Headspace method (HS)

Also known as the *static headspace method*, this method is less sensitive (ppb level) compared to the purge & trap method, but operation is simple, easily automated. The sample is placed in a sealed container, such as a vial, and left at a constant temperature until the gas and liquid phase are in equilibrium. The target substances in the gas phase (headspace) are collected by gas tight syringe. This is injected into the GC/MS. Calibration curve are made by dissolving the target chemicals in purified water, and then treated in the same manner. However, the air-liquid phase equilibrium is very much affected by matrix in which the sample is dissolved, so in a lot of cases standards dissolved in purified water might not be appropriate surrogates from which to prepare calibration curves. In such cases it is necessary to saturate samples with salt, add an external standard and then quantify the target chemicals (Standard addition method). Also, for samples which exist as ions in water the pH must be adjusted. For example, it is easy to analyse fatty acids in alkaline solution, if first the solution containing the fatty acids is acidified by sulfuric acid. It should be noted that pH adjustment is also often needed for the purge & trap method.

(3) Notes on the analysis of volatile compounds

(i) preparation of purified water

One of the most difficult aspects of trace analysis of volatile compounds is to prepare water blanks not containing extraneous volatile compounds. It is common to use commercial mineral water, such as Evian, because it is so difficult to make one's own blank water. In order to make one's own, the following two methods might be tried:

1) purge purified water with high quality helium
2) boil hard ordinal purified water on a gas fire for more than one hour, and then cool down outdoors in an environment where there is naturally no volatile compound contamination place.

(ii) notes for using cryo focus

There are some trapping materials e.g. silica gel or charcoal, which do not remove enough water. As a result, water will freeze at cryo focus. The GC retention times of the target analytes will change unless sufficient water is removed by another method e.g. dry purge, etc.

c) Semi-volatile chemicals

Methods to extract semi-volatile compounds from water include liquid-liquid extraction, solid phase extraction, and recently developed solid phase microextraction (SPME)[2].

(1) Liquid-liquid extraction

Solvent extraction methods use non-polar solvents which are miscible with water to extract the target compound from water by using the greater solubility of the target compound in the solvent than water. Ideally, one selectively extracts the target compound by using a solvent whose polarity is close to that of the target compound. Volatile solvents such as hexane, benzene, ether, ethyl acetate, and dichloromethane are usually used for the extraction of semi-volatile compounds from water. Hexane is suitable for extraction of non-polar compounds such as aliphatic hydrocarbons, benzene is suitable for aromatic compounds, and ether and ethyl acetate are suitable for relatively polar compounds containing oxygen. Dichloromethane has high extraction efficiency for a wide rage of non-polar to polar compounds. Dichloromethane is suitable for simultaneous analysis because of the following advantages: its boiling point is low and easy to reconcentrate after extraction, it is easy to separate from water because of its higher specific gravity, and it is non-flammable. However, dichloromethane, like benzene, is carcinogenic, and recent trends have been to refrain from using these solvents in liquid-liquid extractions. It is sometimes possible to selectively extract semi-volatile compounds from water by changing the character of samples, not changing solvents. For example, by changing the pH of samples, only acid or basic substances can be extracted. When pH of the water is less than 2, basic compounds become fully ionised and are not extracted by the solvent, allowing selective extraction of acidic and neutral compounds.

When extracting compounds which are relatively soluble in water, salting-out techniques are used in order to increase extraction rates. Adding salt to an aqueous sample decreases the solvation power of the solution and the solubility of target compounds. This is useful not only for liquid-liquid extraction but also for headspace and solid phase extraction methods.

Extraction is commonly achieved by shaking the water sample and solvent in a separating funnel. However, occasionally large amounts of emulsion are formed, and it is difficult to separate the solvent from the aqueous phase. If this occurs, the emulsion is often efficiently dispersed (broken down) by adding either a small amount of ethanol, by sonicating the mixture in ultrasonic bath, or by adding anhydrous sodium sulfate, or continuous liquid-liquid extraction can be performed on samples which form emulsions. Continuous liquid-liquid extraction methods repeatedly circulate solvent in special glassware but, although this method has good extraction efficiency, it is not suitable for thermally unstable compounds because the extraction time is long.

(2) Solid-Phase Extraction (SPE)

Solid phase extraction (SPE) is a more rapid, modern alternative to liquid-liquid extraction. SPE is based on the principle that the components of interest are retained on a special sorbent

contained in a disposable mini-column (cartridge). By using SPE one can remove matrix interferences (these either pass through the cartridge or are subsequently washed off) and then isolate with selective enrichment one's target compounds. Solvent use is small. Common cartridges packing materials (solid phases) are charcoal[3),4)] and XAD[5)] resin, silica gel chemically bonded with ODS[6),7)], and high-polymer resin such as polystyrene[8),9)] and polyacrylate[9)]. Cartridges pre-packed with known quantities of adsorbent are on the market, and they are ready to use after simple conditioning.

There are some disadvantages to solid-phase extraction, which are:

- Although solvent use is small, the solvent flow rate affects the recovery rate.
- For samples which include suspended solid (SS), it is necessary to separate SS composition.
- For samples which are heavily contaminated, it is possible to get analyte break through.
- In order to have high and stable recovery rates, it is important to choose the most appropriate solid phase for the target compounds (refer to solid phase manufacturers' manual). One must undertake recovery tests by adding known quantities of standard into the sample matrix, passing such samples through cartridges, eluting the target compounds, then determining the amount of analyte recovered.

(3) Solid phase microextraction (SPME)[2)]

SPME is a method used to both extract and concentrate organic compounds in which a fibre needle attachment which has been chemically coated with a fused silica equivalent to a GC liquid phase, is dipped directly into liquid samples, or exposed to the headspace vapors from liquid or solid samples. Because SPME has only recently been developed, there are few reports of its use with real environmental samples, and we must wait for the results of future investigation before recommending use. However, the fact that organic compounds can be analysed easily and quickly without using any solvents suggests that this is the direction in which the next generation of analytical methods should proceed.

B. Sediment, soil, and biological samples

a) Purge & trap method (P&T, dynamic headspace method)

The purge & trap method can be used to extract volatile compounds from solid samples such as sediment. The sample (less than 1 g) is placed into the purge bottle, suspended in adding water, then treated and subsequently analysed in the same manner as water samples. Samples which include high concentration of VOC are extracted with methanol, and then a part of extract is analysed by purge & trap after being added to blank water.

b) Organic solvent extraction method

There are three organic solvent extraction methods for semi-volatile compounds from solid samples: 1) Soxhlet extraction, 2) extraction after mechanical mixing such as shaking, homogenisation, or stirring, and 3) ultrasonic extraction.

(1) Soxhlet extraction

In soxhlet extraction, organic components in solid samples are extracted from the matrix by continuously washing the solid with a volatile solvent in a specialised piece of glassware (soxhlet extraction apparatus). This is the most common method for extraction of organic compounds from solid samples, and is used as an extraction rate standard for the newly developed extraction method known as supercritical fluid extraction. Non-polar solvents such as benzene or dichloromethane, polar solvents such as methanol, or mixtures of polar and non-polar solvents whose boiling points are close to those of ethanol / benzene, or acetone / hexane are used. Benzene is known to be an especially efficient extraction solvent for PAHs, and acetone for sulphur-containing compounds. However, soxhlet extraction takes long time to get high extraction efficiency, and is not suitable for organic compounds which are thermally unstable.

(2) Methods extraction after mechanical mixing such as shaking, stirring, or homogenization

Shaking and stirring: These methods are in essence derivations of liquid-liquid extraction. Sample extraction is achieved by simply placing solid samples in centrifuge containers with organic solvents and shaking. After extraction, separate sample matrix and extracts by centrifugation or filtration.

Homogenization: This method is suitable for the extraction of non-polar compounds in biological samples. Anhydrous sodium sulfate is added to the sample, the sample is then homogenised in the presence of a non-polar solvent. After extraction, separate sample matrix and extracts by centrifugation or filtration. Extracts usually include lipids, so defatting processes are required before analysis.

(3) Ultrasonic extraction

Ultrasonic extraction uses ultrasonic vibrations to extract samples with polar solvents in an ultrasonic bath. This is often used for chemical extraction from solid samples because it's simple.

c) Steam distillation (including circulating steam distillation)

In steam distillation, steam is introduced into a macerated solid sample. The target analytes are co-distilled out of the sample container with the steam, and are collected after condensation of the steam to liquid water. In the circulating method, the condensate is extracted by liquid-liquid extraction using a non-polar solvent. Steam distillation is used for compounds with a relatively high vapor pressure which are not soluble in cold water. Steam distillation is an operation which has both extraction and separation / fractionation functions. Because it can separate target compounds from non-volatile compounds and sulfur contained in sediments, purification after extraction is easy.

d) Supercritical Fluid Extraction (SFE)

Gases such as CO_2 and N_2O become fluid when temperature and pressure reach a critical point called the supercritical phase. In supercritical fluid extraction (SFE), this fluid is used to extract target substances in solid samples. This is a relatively new method which has recently begun to be applied extensively. Instruments connecting the SFE apparatus and on line GC have been developed in which the series of steps from extraction to analysis have been automated.

The sample is placed in an extraction chamber through which the supercritical fluid is forced, the target substances are extracted from the samples and trapped in vials by small amounts of methanol or ODS resin. The amount of organic solvent used is much smaller than soxhlet extraction.

The fluids used for SFE include CO_2, N_2O, SF_6, methanol, and water. The fluid used is chosen to meet the analytical purpose. The most commonly used is CO_2. However, supercritical CO_2 is very non-polar, so modifiers are added to improve extraction efficiency for polar compounds. Modifiers for CO_2 include methanol, dichloromethane, acetonitrile, water etc. Modifiers are mixed with the CO_2 using a pump, or spiked directly into the samples.

C. Air Samples

Air samples are categorised as being particulate and gas depending on the phase in which the target substances are found in the air. Since sampling methods for the two types of air sample are different, as mentioned in Chapter 2, sample preparation methods are explained for each sampling method.

a) Solvent Extraction from Filter Papers or Adsorbents

Filter paper is used to trap particulates, while adsorbents are used for samples containing substances intermediate between particulates and gases. Extraction methods for target substances trapped on filter papers and adsorbents are those used for solid samples and sediments etc., such as ultrasonic extraction and soxhlet extraction. However, ultrasonic extraction is not suitable for polymer adsorbents, since they are not strong enough. Use soxhlet extraction for such polymer adsorbents and polyurethane foams. On the other hand, soxhlet extraction is not suitable for volatile and thermally unstable substances. For such compounds, and other adsorbents use ultrasonic extraction. Changing solvents to meet the requirements of different adsorbents or target substances can make for more efficient extraction. When using a polar solvent such as acetone, add salt water after extraction, and then extract by liquid-liquid extraction with a hydrophilic solvent.

b) Thermal Desorption

Adsorption followed by thermal desorption can introduce the whole trapped target substances into GC / MS. This makes such methods suitable for analysing volatile compounds, and samples containing low concentrations of target analytes where it is not easy to be collected large amounts of sample. The basic scheme is shown in **Figure III-1-3**. Adsorbed compounds are thermally desorbed by heating the adsorbent material with a heater. Changing the direction of flow of the GC carrier gas by using a three-way valve allows introduction of these compounds into the GC column. Equipment in which all procedures are automated is on the market. Such equipment generally has a cold trap, where desorbed samples are temporarily trapped in a concentration tube (cold finger) cooled by liquid nitrogen, and then introduced into the column by heating. Such cold trap functions make sample introduction quicker, and gives sharper peaks.

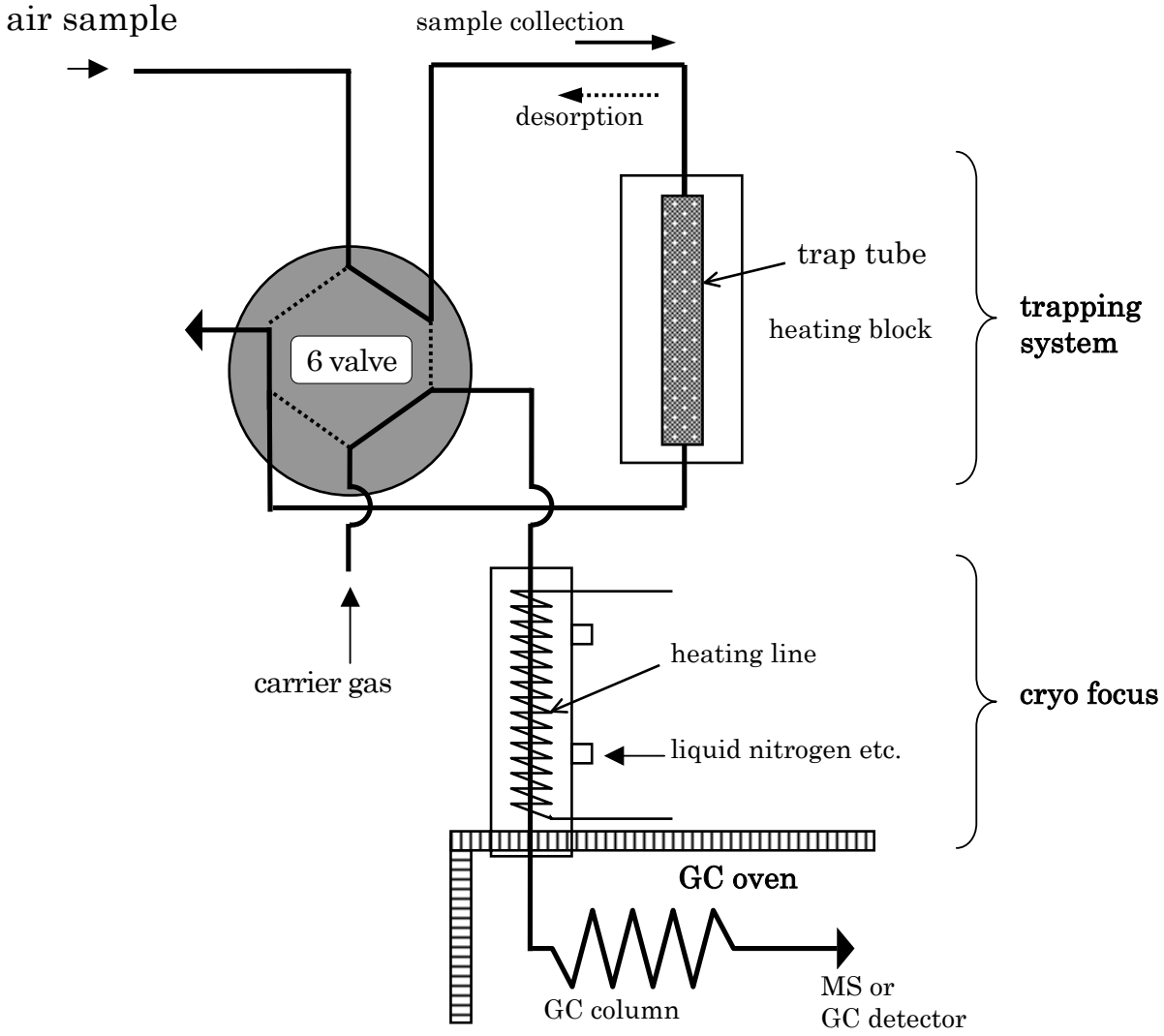


Figure III-1-3 Flow direction in adsorption trap-thermal desorption method

III.1.2.2 Dehydration Methods

Because extracts from liquid-liquid extractions often contain water originating from the sample, sample concentration or clean-up using columns cannot be done directly. Thus, it is necessary to dehydrate the extract. Dehydration is most commonly achieved by using anhydrous sodium sulfate. Samples are dehydrated by adding the anhydrous sodium sulfate directly to the extract and then filtering the dry solvent solution, or passing the samples through a column or funnel packed with anhydrous sodium sulfate.

III.1.2.3 Concentration

Use a Kuderna-Danish (KD) concentrator or rotary evaporator to concentrate the extract or column chromatography eluant. Whether the KD method or rotary evaporation is chosen depends on the boiling point of the target compounds, their sublimation character, timeframe for analysis etc.

A. KD Concentration

KD concentration takes longer than rotary evaporation, but there is less loss of target chemical through evaporation, and this method is as applicable to low boiling compounds as to high boiling compounds. This method is able to concentrate samples down to a few mL. For further concentration, one must use a micro-Snyder column or evaporate under a stream of nitrogen. Evaporating under nitrogen gas may cause evaporative loss of low boiling point compounds. However, the micro-Snyder column method can concentrate samples containing low boiling point compounds to volumes of 0.5 mL.

B. Rotary Evaporation

Rotary evaporation can concentrate large volumes of samples in a relatively short period of time. However, it has big evaporative losses and is not suitable for low boiling point compounds.

III.1.2.4 Clean-up Method

Extracts from environmental samples can be complicated mixtures. Components of these mixtures can interfere with GC and HPLC analysis by giving poor separation because of over separation capacity of a column or by containing compounds that elute at the same time as, and interfere with, the target compounds' peaks. Also large amounts of non-volatile compounds or polar compounds can contaminate GC injection ports and columns, which in turn causes problems with analysis. It is, therefore, necessary to clean up, or remove, non target compounds as much as possible. Clean-up methods include acid - alkaline partition, acetonitrile - hexane partition, and column chromatography. Column chromatography separation can involve adsorption, partition, gel permeation, ion exchange etc. The following section describes clean-up methods usually used for environmental analysis.

A. Adsorption Chromatography

Adsorption chromatography is able to separate the constituents of complex samples according to equilibrium between adsorbent (stationary phase), solute (chemicals) and eluent (eluting agent). Generally normal phase chromatography is used. Samples are dehydrated and concentrated, then added to the column. The column is eluted first with a non-polar solvent such as hexane, and then the polarity of the solvent is gradually increased. This has the affect of progressively eluting more polar constituents. Adsorption chromatography is most suitable for the separation of relatively non-polar compounds. Adsorbents typically used are alumina, silica gel, and florisil. The relative strength of adsorption of these materials is: silica gel < florisil < alumina. The suitability or nonsuitability of these materials for the target compounds depends on the strength of adsorption. To find the most appropriate adsorbent, refer to published papers and actual tests. Perform actual sample analysis after confirming adsorption activity level, composition of eluent, and elution pattern because there are some compounds which decompose in the commonly used eluents, such as diethyl ether or acetone.

a) Silica gel

Silica gel is weakly acidic, amorphous silica. Activate this adsorbent by heating at 150 - 160 °C for several hours. It is common to contain 3 - 5 % by weight water in the adsorbent because completely activated silica occasionally does non-reverse adsorption. This material is useful for compounds containing most ionic and non ionic functional groups. However, one must be cautious when using eluents which contain methanol or ethanol because they decrease the adsorbent's adsorption activity.

b) Florisil

Florisil is the commercial name for a magnesium silicate with an acidic character. This material is available on the market from Floridin Co. in a form which has been activated at 667 °C. This material is mostly used for clean-up of samples for GC containing pesticides, organochlorine compounds, esters, ketones, phthalic esters, nitrosoamines, organophosphate pesticides (which include a phosphorus - oxygen bond which can decompose on florisil), separating aliphatic and aromatic hydrocarbons, etc. However, florisil's activity varies between batches, one must watch out for changes in elution patterns. In addition, it is known that some pesticides decompose in ethylether on florisil.

c) Alumina

There are three types of alumina, basic (pH 9-10), neutral, and acidic (pH 4-5). Alumina is used after dehydration at 400 - 450 °C. Water is added to the alumina to adjust activity. Basic

alumina has the strongest adsorption capacity, and is used for basic and neutral compounds, such as alcohols, hydrocarbon, steroid, which are stable in alkaline media. However, ethyl acetate cannot be used as an eluent because esters are unstable in alkaline media and decompose. Acetone cannot be used as an eluent because it causes amidol condensation and produces diacetone alcohol. Neutral alumina has lower activity compared to basic alumina, and is used for aldehydes, ketones, quinones, esters, etc. Acidic alumina is suitable for acidic pigments (dyes) or acidic compounds which are adsorbed by basic and neutral alumina.

B. Partition Chromatography

Partition chromatography is able to separate the constituents of complex samples according to partition equilibrium between a non-polar solid phase and a polar solvent (in this case separation is by *reverse-phase* chromatography). The non-polar solid phase is packed into a column, the sample mixture dissolved in water and loaded onto the column, then the components of the mixture, from most polar to least polar, are eluted with solvent mixtures such as water - methanol, or water - acetonitrile. This method is, therefore, most suitable for separation of high polarity compounds. The most commonly used solid phase is ODS, which has an octadecyl function bonded to the silica particle. This material is commonly used for the fractionation of samples by HPLC.

C. Gel Permeation Chromatography

Gel permeation chromatography separates molecules by size. Compounds are separated when sample mixtures are passed through a column packed with material of a known pore size. Larger molecules elute faster. Gel permeation chromatography is used as a general separation method for semi-volatile compounds. However, the separation ability is worse than other chromatography methods, so GPC is generally used to remove lipids, proteins, and natural resins from samples, not for detailed fractionation.

D. Ion-Exchange Chromatography

Ion exchange chromatography is used to separate compounds which have fully ionisable function groups. Samples are loaded onto the top of a column packed with an ion exchange resin, and eluted by an electrolyte solution. Which ions are separated and the order they are eluted depends on the ion exchange- adsorption character of the molecules with the resin.

E. Acetonitrile - Hexane Partition

Acetonitrile - hexane partition is commonly used to remove lipids from sample extracts.

Target compounds are partitioned into the acetonitrile phase, and lipids partitioned into the hexane phase. However, when the target compound's polarity is extremely small, the target compound too can partition into the hexane phase.

F. Acid - alkaline Partition

Acid-alkaline partition is used to separate neutral, acidic, and basic components by adjusting the pH of aqueous samples. Compounds such as phenol can be extracted into organic solvent when aqueous samples are made acidic (below pH 2). Next, phenols in the extract are reverse extracted by water of pH is 12 - 13. Finally, this aqueous phase is acidified (to below pH 2), and re-extracted by organic solvent. In this manner, only phenols can be extracted from samples. Basic compounds such as amines can also be separated by pH reversal.

G. Sulfur-Cleanup

Elemental sulfur is often found in anaerobic sediment samples. Elemental sulfur can be dissolved in a number of solvents, since its solubility is quite similar to organochlorine and organophosphate pesticides. Sulfur is eluted in the first fraction eluting from florisil clean-up columns. When analysing samples which include sulfur, unless sulfur is removed, an interference peak caused by sulfur is often found between the solvent peak and that of aldrin. To remove sulfur, stir sample solutions well with either copper powder, mercury, or tetrabutyl ammonium solution. Since some compounds which contain sulfur or phosphorus, such as phosphates or pesticides, are unstable, one must check these compounds' stability before treating the samples with sulfur removing materials.

H. Alkaline Decomposition

Alkaline decomposition is used to extract compounds which are stable in alkaline media, such as PCBs, from biological samples which contain lipids. Such samples are refluxed in alkaline ethanolic solution. This saponifies lipids. Salt solution is added to the sample, and the target compounds are extracted by liquid-liquid extraction. It is possible to remove lipids and extract target analytes at the same time using this method.

III.1.2.5 Derivatisation

Derivatisation involves chemically modifying the target compounds. It is common to convert hydroxyl group (phenols), carboxyl groups (fatty acids), amino groups (amines), and organometallic

compounds (organotins) into their trimethylsilyl, ester, acyl, and alkyl derivatives. By derivatising such chemical functional groups, one can make non-volatile compounds volatile. Thus one can analyse by GC compounds which normally cannot be analysed by GC, including compounds which are normally adsorbed by separation columns. It is, however, possible to analyse some polar compounds without derivatisation if one uses a fused-silica capillary column. One should not rush to derivatives target compounds because there are problems with derivatisation which must be carefully considered, such as yield of derivatisation, derivative recovery, stability of a derivative, identification and confirmation.

A. Esterfication

Methyl ester derivatives are generally stable, isolation is usually easy, and it is often possible to confirm the yield and stability of reaction products. After methylation, the increase in molecular weight of the methyl ester derivatives is small, some show molecular ions in mass spectra, and one may therefore use data reference systems. However there are some problems, for example, methylating reagents are often not highly reactive, and many don't react with alcoholic hydroxyl groups and amine groups, although they are used for fatty acids.

B. Silylation

Trimethylsilylation (TMS) shows the highest reactivity of all derivatisation methods for most compounds which have active hydrogens. However, this method should only be chosen when methylation and acetylation cannot be used, since there some disadvantages:

1) products of silylation are easily hydrolysed and cannot be stored for long periods
2) products of silylation often cannot be isolated, and therefore reaction solutions often have to be analysed directly by GC
3) injection of reaction solutions into GC tends to make GC 'dirty' because inorganic silicone compounds are formed
4) polyethylene glycol (PEG) columns react with silylating reagents.

C. Acetylation

This is one of the most often used derivatisation methods, because acylating agents react well with many functional groups, such as alcohols, thiols, and amines. Fluoroacetylation, particularly heptafluoropropionylation, is often used because highly sensitive analysis by ECD or MS is possible.

D. Other Derivatisation Methods

Pentafluorobenzylhydroxylamine (PFBOA) and 2,4-dinitrophenylhydrazine are used for derivatisation of aldehydes. Also "Grignard" reagent is used for alkylation of organotins[10].

III.1.3 Instrumental Analytical Methods

Only GC / MS is discussed hereafter because of the high sensitivity, high selectivity, universality, and the large amount of information available on what is often considered the most appropriate analytical instrument for environmental analysis.

III.1.3.1 GC/MS Analysis

It is not too much to say that whether trace chemical analysis by GC / MS is successful or not depends on whether the set-up of the GC is appropriate for the target compounds or not. Recently because many analyses use fused-silica capillary columns, there has become less need to pay attention to the selection and inactivation of columns (*cf.* when packed columns were used). However, in order to analyse chemicals at sub-ng concentrations efficiently and accurately, it is still necessary to carefully choose columns, sample injection conditions, column temperature conditions, etc. Selection of quantifying ions directly affects MS identification and detection limit. Here follows some basic information which should be known when trace chemicals are to be identified and quantified by capillary GC / MS and things which should be considered when undertaking GC/MS.

III.1.3.2 Factors Affecting Capillary GC Analysis

Good separation in the shortest period of time is the ideal (optimum) for good GC analysis. These criteria are related to the liquid phase of columns, film thickness, length, internal diameter, and temperature.

A. Selection of capillary column

a) Selection of liquid phase of capillary column

Capillary columns are available with many kinds of liquid phase, from non-polar methylsilicones to highly polar polyethylene glycols. For the analysis of most compounds it is sufficient to use four types of liquid phases - methylsilycones, phenylmethylsilicones, cyanopropyls, and polyethylene glycols. Selection of the most appropriate liquid phase, and hence column, to

separate target compounds is dependent on partition coefficients. For instance, to separate non-polar components such as n-alkanes, use non-polar columns - these have the largest partition coefficients for such components. Highly polar liquid phase columns, such as PEGs, are suitable for analysis of highly polar compounds such as alcohols. However, polar columns (especially PEGs) are often thermally unstable and oxygen sensitive, and often have problems with column bleeding. As a result, their operating temperature is limited compared to non-polar columns. These disadvantages make columns with highly polar liquid phases difficult to use. Therefore, if a target chemical shows a sharp peak with no tailing peak, one has to choose a column with a lower polarity liquid phase. Choose columns by referring to analytical method information supplied in column suppliers' catalogues and published analytical methods.

b) Column length

The length of a column length directly affects separation efficiency and analysis time. Although separation efficiency is proportional to the square root of column length, it is not recommended to use very long columns in order to improve separation because analysis time is proportional to column length. For example, when a 30 m column is exchanged for a 60 m column, separation improves 1.4 times but analysis time is doubled. However, simply cutting a few meters off the end of a dirty column doesn't make a big difference to separation efficiency.

c) Column internal diameter

The internal diameter of a column affects column efficiency, retention character, and the amount of sample that may be loaded onto the column while still obtaining analyte separation. The smaller internal diameters, the better separation efficiency, but the less sample may be loaded onto the column. For environmental analysis, column bore of internal diameter 0.2 - 0.35 mm is generally used. Columns whose internal diameter is about 0.25 mm give good separation, although one can load larger samples onto columns whose internal diameter is about 0.32 mm. Wide bore columns, i.e. with an internal diameter of more than 0.5 mm, can be used as substitute for packed columns because the amount of sample that may be loaded is usually several μg for each constituent. However, such columns cannot be used for GC/MS unless the GC/MS has a large vacuum pump (and even so regular bore column resistant tubing is needed), or an enricher (apparatus which separates helium at the interface between the GC and MS).

d) Film thickness

The film thickness affects the column's relative character and amount of sample that may be loaded onto it. The thicker film is, the larger the sample that may be loaded onto the column. Columns with thicker films are suitable for the analysis of low boiling point compounds because

solute constituents are held strongly by the column. Conversely, thinner columns are suitable for the analysis of high boiling point compounds (analysis of high boiling point compounds using thicker film columns requires higher column temperatures, which in turn causes increased column bleeding). For GC/MS, columns should be used which have little column bleeding and unnecessarily thick film columns and non chemical bonding type columns should be avoided. Because small internal diameter and thin film columns can only be loaded with small amounts of sample, it is necessary to take measures to prevent peak shape abnormalities, such as leading, by minimising the amount of sample loaded onto the column or by using a different column.

e) Basic columns

In light of the factors outlined above, the following columns should be considered for chemical analysis of environmental samples:

- For volatile compounds: liquid phase, methylsilicone; length, 60 m; i.d., 0.32 mm, film thickness, 3 μm.
- For semi-volatile compounds : liquid phase, methylsilicone or 5% phenyl 95% methylsilicone; length, 25 - 30 m; i.d., 0.2 - 0.32 mm; film thickness, 0.1 - 0.5 μm.

B. Sample Injection Methods

For capillary columns, there are three principle requirements for any injection method, namely to introduce samples as a narrow width band, to not change sample composition, and to have good reproducibility. Common sample injection methods recently are direct injection, split/splitless injection, and on-column injection methods. In order to select the method that is most suitable for the samples in hand and meet the above criteria, one must understand some of the key features of these methods.

a) Direct injection

In direct injection methods, the whole sample is introduced into the vaporising chamber. This method is used for widebore columns whose internal diameter is more than 0.5 mm, or packed columns. The injection port temperature is typically 20 - 30 °C higher than the GC oven (column) temperature for iso-thermal analysis, and also typically 20 - 30 °C higher than the final oven temperature when temperature gradient programming is used.

b) Split / splitless injection

Split and splitless injection methods can be used at the same injection port.

- **Split method:** In this method, the sample is vaporised instantly in the gas chamber, then a narrow sample band is introduced into the column by introducing only a small part of the

vaporised samples and discharging the rest. This is a useful method for samples with high concentrations of target analytes. It is not suitable for samples with low analyte concentrations such as environmental samples. Also, when analysing compounds which contain a mixture of components with a mixture of boiling points (low, medium, high), such as petrol, fractional distillation (discrimination) may occur in the vaporisation chamber. To prevent this, increase the temperature of the vaporisation chamber, and increase the system's heat capacity putting deactivated glass wool into the insert (injection liner).

- **Splitless method** : In this method, the sample, including any solvent, is introduced onto the column through a modified heated vaporiser. Solvent is removed from the sample by venting the injection to the atmosphere when most of the solvent and essentially all of the sample have entered the column - perhaps 30 sec to 2 min. The correct lag time before venting is critical since too little time causes loss of sample, too long a time period causes a solvent peak larger than necessary and which in turn may cause spectral interference. This method is suitable for environmental samples because it is able to introduce samples with low target analyte concentrations quantitatively into columns. In addition, relatively non-volatile compounds, which often cause damage to columns, are not volatilized and therefore do not penetrate far into the column. Chambered injection port liners are used with the splitless method because this method doesn't need to vaporize samples (*cf.* split method).

c) On-column injection

In on-column injection, the sample solution is injected onto the column as liquid, and then the column temperature is increased. This procedure makes this injection method suitable for the analysis of thermally unstable compounds. This is suitable for trace analysis because the whole sample is injected onto the column. However, if contaminated samples are injected, the column's useful working life span is shortened because in this case relatively non-volatile compounds, which often cause damage to columns, are volatilized and can penetrate far into the column. Therefore, it is usual to attach a guard column to the separation column to prevent column contamination.

Recently the Septum Programmable Injector (SPI) has become popular. This equipment has improved cool on-column methods since with it is possible to control the injection temperature independently of the oven's cooling and heating cycles. It is thus possible to setup injection conditions optimised for producing narrow sample band-widths.

III.1.3.3 Mass Spectrometry

There are two ways to use mass spectrometers in scanning mode or selected ion monitoring mode (SIM). In order to select the appropriate operating mode, one must understand some of the key features of these methods.

A. Scanning method

When operated in the scanning mode, the mass spectrometer records the mass spectrum of all ions detected within a defined mass range by scanning at predetermined mass (m/z) intervals. The record of total ion intensity on each scan with time is called the total ion chromatogram (TIC). The TIC is equivalent to, for instance, a GC-FID chromatogram. The data making up the TIC is recorded electronically, and specific m/z information can be extracted and printed. These latter are called mass chromatograms. The scanning method can change identifying ion freely after analysis, and there is often no need to re-analyse the sample even if there are interfering peaks.

The accuracy and reliability of analyte identification is significantly better than SIM, because one can obtain the mass spectrum of the target compound using the scanning method. However, the method's sensitivity is theoretically much worse than SIM (several to several tens of times) in the case of magnetic field type quadrupole mass spectrometers - low resolution MS cannot perform trace analysis. Finally, the amount data collected is extremely large compared to SIM.

B. SIM method

When operated in the SIM mode, the mass spectrometer monitors specified ions with high sensitivity. This is done by setting up electric or magnetic fields (depending on the instrument design) which focus only ions of a predetermined m/z ratio on the detector. A SIM chromatogram equivalent to the mass chromatogram obtained by the scanning method can also be obtained. As mentioned earlier, the sensitivity of SIM operation is better than the scanning method because the time spent on analysis of the target analyte ions is longer. Recent GC/MS systems have been developed that can routinely detect picogram (pg) levels of organic compounds. However, the number of ions which can be measured at any one time is generally limited to ten. This means the number of compounds which can be analysed at any one time is generally limited to five (at least ten sampling points for each GC peak are needed to get an accurate area for the GC peak. Therefore, if 2 ions per compound are monitored, the number of compounds which can be measured at the same time is limited to a maximum of five). In order to overcome this limitation, a Grouping method which changes the measured ion depending on GC retention time is used.

The accuracy and reliability of analyte identification is lower compared to scanning method because the number of ions measured is limited. Selection of ions to be monitored is important in

order to get high sensitivity and high reliability of identification. Usually multiple ions per compound are measured, and it is necessary to confirm the compounds from relative isotope intensity ratio.

a) Selection of quantifying ion and confirmatory ion for SIM method

In principle one tries to select ions with high sensitivity and high selectivity. First, measure the accurate mass spectrum of the target compound, confirming compound identity by comparison with library spectra. When the intensity of the target compound's molecular ion (parent ion) is high, make that ion the quantifying ion. If the molecular ion is small, choose an ion of high mass number (m/z) and high intensity as the quantifying ion (ions whose mass number (m/z) are less than 100 should be avoided). Choose a confirmatory ion (an ion known to be derived from the target compound and which proves the presence of the target) of high mass number (m/z) and high intensity. Compounds which contain chlorine or bromine produce isotope ions (molecular and fragment ions containing the chlorine or bromine isotopes) in the same ratio as the ratio of naturally occurring chlorine or bromine isotopes. These isotope ions are characteristic and the number produced depends on the number of chlorine or bromine atoms within the molecule. Isotope ions are ideal as confirmatory ions. After deciding which ions to monitor, measure standard solutions, and check if the desired is achieved, if the background noise is small compared to the signal intensity (S/N ratio is fine), or if there are no interferences, etc. If such parameters cannot be achieved, change the measurement ions.

III.1.3.4 Methods for the Calculation of Concentration

A. External Standard Method

The external standard method uses the relationship between peak areas (or height) and analyte concentration. The calibration curve is constructed after multiple, identical injections of a number of standard solutions of known concentration into the GC/MS.

Calibration curves for wide (mega) bore columns can be constructed after manual injection of samples, since several μL of samples is injected at one time and the variation in injection volume is small compared to the total volume injected. However, for regular bore columns, use of an autosampler is desirable, since the volume of sample injected is small and autosamplers are capable of very precise injection of small volumes.

B. Internal Standard Method

The internal standard method can be applied to every type of column. Standard solutions of

the target analyte are prepared in the same way as if using the external standard method, but to each is added a known amount of an internal standard. The solutions are measured. After measurement, calibration curves are constructed using the relationships between target analyte peak intensity and that of the internal standard (target compound peak intensity: internal standard peak intensity ratio) and target analyte concentration and that of the internal standard (ratio of target compound concentration: internal standard concentration). When determining target analyte concentrations in real samples, add the same amount of internal standard to the sample as added to the standard solutions, then calculate the concentration of target analyte from the target compound peak intensity: internal standard peak intensity ratio.

When using the internal standard method, it should be noted that the relative standard deviation (coefficient of variance) of ratios of peak intensities generally becomes larger as the separation (difference in retention times) of the internal standard and the target compounds increases. Therefore, a compound whose retention time is close to that of the target compound should be used as the internal standard, and when many target compounds with wide ranging retention times are being investigated, use of multiple internal standards is desirable. Finally, the chosen as internal standards should obviously not be found in the samples, and should be sufficiently stable to allow analysis by GC/MS. Internal samples synthesized using stable isotopes, such as deuterium or ^{13}C, are ideal compounds.

C. Relative Response Factor method

When a large number of compounds are being investigated simultaneously, it becomes difficult to make calibration curves for each measurement. In such cases, use the relative response factor method. This method is a variant on the internal standard method. First, make calibration curves (a minimum of five points from the detection limit of the instrument to the highest necessary concentration) in the usual manner. Then find linear range of calibration curves for each compound, and calculate the sensitivity against internal standard within the linear range using the following formula.

$$\text{RF of the compound} = \frac{\text{quantifying ion intensity of the compound} \,/\, \text{injection amount of the compound}}{\text{quantifying ion intensity of internal standard} \,/\, \text{injection amount of internal standard}}$$

When using the relative response factor method for sample analysis, analyse a standard solution whose concentration is near the centre of the linear range of calibration curve, or between a standard value and the determination limit, before sample analysis, and check if prescribed result can be obtained (for example, determined value is within ± 30% of the actual concentration). If the prescribed result cannot be obtained, it may be because of functional deterioration of the GC/MS, or decomposition of the target compound in the standard solution. Only begin analysis of the actual samples after solving the problem.

When analysing trace chemicals below the nanogram (ng) level, the smaller injection amount is, the smaller the RF value is. That is, the calibration curve becomes flatter. This symptom is especially notable when the polarity of target compound is increased. In this case determine using not the average RF value but RF value of the concentration which is close to the detection level.

D. Standard addition method

The method of standard additions may be used when components of the sample matrix influence analysis by producing physical or chemical interferences which cause calibration curves to differ from those made using purified water. To use the method of standard addition, first determine the analyte concentration in the samples, then add a small amount of a standard analyte solution of known concentration to the sample, and the analysis is repeated using identical reagents, instrument parameters and procedures. Readings must be corrected for any background signal. The result should be checked by repeating this procedure with at least one other standard addition. By this means, a plot such as **Figure III-1-4** may be obtained, from which the in the samples can be determined.

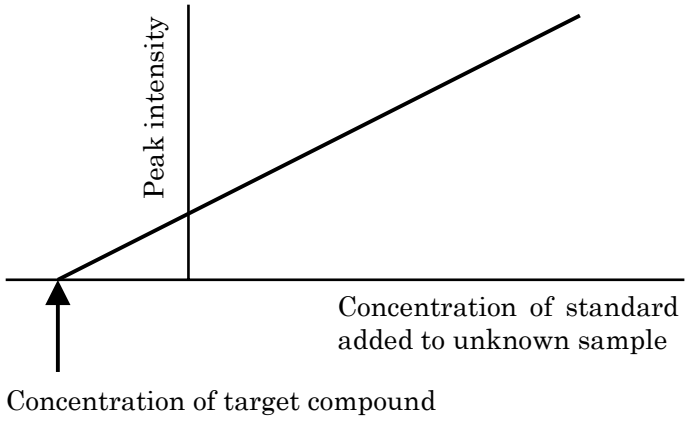


Figure III-1-4 Calibration curve for standard addition method

III.1.4 Procedures for analysis of new compounds

The steps typically required when analysing for the first time compounds whose analytical method is known are as follows.

(1) Collect as mush information as possible from the literature on such things as analytical methods, manuals, physico-chemical characters of the target compounds.

(2) Obtain pure target compounds, internal standards and / or surrogate compounds, and make stock standard solutions of about 1000 mg/L. As mentioned earlier, use as an internal standard a compound whose GC retention time is close to that of the target compound under the anticipated GC conditions, which is stable, and which gives a stable GC peak. Polyaromatic compounds labeled with stable isotopes will be ideal. Also as mentioned earlier, use as a surrogate compound a compound whose physico-chemical character is similar to that of the target compound. Again, target compounds labeled by stable isotopes are ideal. For both internal standards and surrogate compounds, use compounds which are not found in the environment under investigation.

(3) Selection of a GC column. Select a column based on information found in literature analytical methods or manufacturer's catalogues.

(4) Measurement of mass spectrum of target compounds and internal standards. Measure mass spectrum of target compounds, surrogates, and internal standards. The amount of material to be injected is the amount which will definitely give a good mass spectrum (about several ng). At this stage, it is efficient to increase the GC oven temperature from a low temperature because there is no need to repeat measurement. If it is difficult to tell which peak is the base peak because too much material was injected, decrease the amount of material injected and remeasure.

(5) After confirming, by analysis of the collected mass spectrum or by using the instrument's library spectra-matching system, that the mass spectra collected are correct or not, decide which ions will be used for determination and confirmation. Also, finalise GC oven programming to ensure the best measurement conditions in the shortest time.

(6) Prepare a series of low concentration standard solutions for making calibration curves, and make calibration curves of target compounds and surrogates, if used. The amount of internal standard to be used will equate with the minimum amount of compound which produces a stable peak area (0.1 - 1 ng is appropriate). Next determine the instrument's minimum detection limit. This minimum detection limit is the value at which it is possible to measure accurately and precisely the peak area (MS response) of the targets even if the GC/MS system deteriorates. A minimum signal to noise (S/N) ratio of about 10 is required. If the monitor ion is interfered with, or the S/N ratio is bad, change the monitor ion and

remeasure.

(7) Confirm the linear range of the calibration curves from instrumental detection limit to the highest concentration. Determine the volume of a sample and final concentrate for GC/MS analysis which can be analysed in the shortest possible time, while still satisfying the survey's purpose and required detection limit, and using the least solvent.

(8) Determine the recovery and elution pattern by means of the necessary preliminary analytical experiments, such as column chromatography. The concentrations of target compounds used in such preliminary experiments will be that quantity which can be determined accurately and precisely.

(9) Conduct recovery tests, passing spiked reagent water through the full analytical process using identical reagents, instrument parameters and procedures. Conduct at least four recovery tests, as well as a blank. The amount of compounds spiked is generally several times the detection limit.

(10) Evaluate the results of overall recovery tests. Calculate the average recovery and the relative standard deviation (RSD). For water samples, it is acceptable if the recovery is more than 80 % and the RSD is less than 20 - 30 %.

(11) Conduct recovery tests using typical actual samples. The concentrations of spiked analytes should be the same as that found in the real samples (if known to contain target compounds), otherwise the concentration should be several times the detection limit. Blank samples are real samples without addition of compound.

(12) Confirm that the monitor ions are not subject to interference by matrix constituents, and that the recovery of the target analytes when spiked into the sample matrix is almost the same as the results of recovery tests using reagent water.

(13) Conduct actual analysis. Don't forget to use prescribed quality controls.

III.2 Practical analytical methods

This chapter summarises some practical methods for the analysis of chemicals in the environment using GC/MS.

III.2.1 Simultaneous analytical method for toxic chemicals in water and sediment using GC/MS

III.2.1.1 Target compounds

Hexachlorbenzene (HCB), dieldrin, p,p'-DDT and its metabolized products (p,p'-DDD, p,p'-DDE), chlordanes (trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor), HCHs (α-HCH, β-HCH), terphenyls (o-terphenyl, m-terphenyl, p- terphenyl), BHT, benzo(a)pyrene, tributylphosphate

III.2.1.2 Summary of analytical methods

Water samples : add surrogates, extract with hexane, dehydrate, and concentrate. Finally, determine analyte concentrations by GC/MS-SIM.

Sediment samples : add surrogates. Perform acetonitrile-hexane partition by first extracting with acetonitrile, then adding water to the acetonitrile layer, and finally extracting with hexane. Dehydrate, concentrate, and partition by silica gel column chromatography. Finally determine analyte concentrations by GC/MS-SIM.

III.2.1.3 Detection limit

For the purposes of most environmental surveys and most chemicals, the minimum detection limits using these analytical methods will be approximately 0.01 ng/mL for environmental waters, and 1 ng/g (dry weight basis) for sediments.

- For aqueous samples, conduct addition and recovery tests seven times, by adding target compounds into blank samples (samples of the same / similar matrix which don't contain the target compounds) at concentrations 3 to 5 times the lower end (minimum value) of linear portion of the calibration curve, and then calculate detection limit from the following equation.

$$DL=t_{n-1} \times S$$

where t_{n-1} is the value of student t-test (99% confidence level, or 3.143 when measurements are undertaken 7 times), and S is the standard deviation of the results of the 7 measurements

- For sediment samples, calculate the minimum detection limit in the same manner as for aqueous samples (above) , by adding target compounds into blank samples (samples of the same / similar matrix which don't contain the target compounds) so as to give final concentrations in the range 2 - 5 ng/g (dry weight basis).
- When the minimum detection limit is higher than the value required by the environmental survey's purpose, two strategies may be followed which may achieve the desired detection limit : increase the instrument's (GC/MS) capacity, or increase the size (volume or mass) of the sample extracted. This latter step increases the concentration of target analyte in the solution injected into the GC/MS.

III.2.1.4 Reagents and apparatus

A. Reagents

- Organic solvents : pesticide analysis grade
- Target compounds : commercial standard reagents
- Surrogates (HCB-$^{13}C_6$, p-terphenyl-d_4, benzo(a)pyrene-d_{12}) : commercial standard reagents
- Internal standards: commercial standard reagents, such as naphthalene-d_8, fluoranthene-d_{10}, perylene-d_{12}
- Anhydrous sodium sulfate : pesticide analysis grade or reagent special grade, heated at 700 °C for 8 hours then cooled slowly in a desiccator
- Sodium chloride : pesticide analysis grade or reagent special grade, heated at 700 °C for 8 hours then cooled slowly in a desiccator
- Purified water : wash twice with dichloromethane, and finally with hexane
- Purified 5% sodium chloride solution : wash twice with dichloromethane, and finally with hexane
- 5% hydrated silica gel : activate "Wako gel C-200," or equivalent, at 130 °C overnight, then cool slowly in a desiccator. Place 100 g of the silica gel into a Erlenmeyer flask fitted with a glass stopper, add 5 mL of purified water, putting the stopper in, and leave for 4 -5 hours. Shake occasionally until evenly mixed.
- Purified charcoal : wash "Darco G-60 charcoal," or equivalent, with benzene, then wash with

acetone and then benzene again. Filter through glass fibre filter, and wash with a small amount of acetone. Air dry, then dry in an oven at 130 °C, and then grind to a powder in a mixing bowl. Dry again in the oven at 130 °C, then finally store in a desiccator.

- Reduction copper : reduction copper for analysis of organic elements (60 - 80 mesh). Store under nitrogen gas, and wash with the solvent being used in the experiment immediately before use.

B. Apparatus

- Silica gel columns for water samples : use a glass column (length, 30 cm; internal diameter, 1 cm) packed with 1 g of 5% hydrated silica gel. Use hexane to add the silica gel as slurry. Lay 2 cm of anhydrate sodium sulfate onto the top of the silica gel.
- Silica gel columns for sediment samples : use a glass column (length, 30 cm; internal diameter, 1 cm) packed with 5 g of 5% hydrated silica gel. Use hexane to add the silica gel as slurry. Lay 2 cm of anhydrate sodium sulfate onto the top of the silica gel.
- Activated charcoal column : use a glass column (length, 30 cm; internal diameter, 1 cm) packed with 10 g of anhydrous sodium sulfate which contains 2.5 % activated charcoal. Use hexane containing 30 % acetone to load the packing material into the column. Lay 2 cm of anhydrous sodium sulfate onto the top of the packing material.
- Kuderna-Danish (KD) sample concentration apparatus
- Separating funnel
- Ultrasonic irradiation instrument (ultrasonic bath is also OK)
- Centrifuge : use a capable of spinning a 100 mL centrifuge tube at 3,000 rpm
- Gas chromatograph / mass spectrometer (GC/MS) : magnetic field type or quadrupole type with data analysis apparatus, and which have prementioned function.

III.2.1.5 Experimental

A. Sample Preparation (note 1)

a) Water samples

Add 50 g of sodium chloride and the prescribed amount of surrogate (note 2) to 1 L of water sample and mix well. Add 50 mL of hexane and shake for 10 minutes. Separate the hexane layer. Repeat this extraction twice, combine the hexane layers, dehydrate with anhydrous sodium sulfate, filter, then concentrate (reduce) the hexane solution to 5 mL using the KD concentrator. Add the internal standard, then concentrate further to 1 mL by evaporating the solution under a stream of nitrogen. Finally, introduce the concentrated extract into the GC/MS for analysis (note 3).

b) Sediment samples

Put 50 g of wet mud into a 100 mL centrifuge tube, add the prescribed amount of surrogate and mix well. Add 50 mL of acetonitrile and shake for 10 minutes. Conduct an ultrasonic extraction of the mixture by placing the sample in the ultrasonic bath for 10 minutes. Centrifuge the sample at 3000 rpm for 10 minutes, and collect the supernatant liquid. Repeat this extraction procedure three times. Combine the acetonitrile extracts. Place the extracts in a 200 mL separating funnel. Saturate the extracts with hexane by slowly dripping hexane into the mixture. Then add a further 10 mL of hexane and shake for 5 minutes. Collect the acetonitrile layer. Add 20 mL of an aqueous solution of acetonitrile (5 % water, 95% acetonitrile) to the hexane layer. Shake. Separate the acetonitrile layer.

Combine the two acetonitrile extracts, and add this mixture to 500 mL of 5 % sodium chloride solution in a 1 L separating funnel. Add 50 mL of hexane and shake for 5 minutes. Collect the hexane layer. Keep the aqueous phase. Wash the hexane layer with 20 mL of 5 % sodium chloride solution. Collect the hexane layer. Combine the aqueous phase with the aqueous phase collected in the previous step. Extract the combined aqueous phases with another 50 mL of hexane. Collect the hexane layer. Wash this hexane layer with 5 % sodium chloride solution, then combine with the hexane layer collected in the previous step. Dehydrate this hexane solution with anhydrous sodium sulfate, then concentrate (reduce) the hexane solution to 5 mL using the KD concentrator. Add the internal standard, then concentrate further to 1 mL by evaporating the solution under a stream of nitrogen. Finally, introduce the concentrated extract into the GC/MS for analysis.

B. Sample Clean-up (note 4)

a) Water samples (note 3)

Load the concentrated solution obtained after KD concentration in part III.2.1.5 A (a) onto a silica gel column. Elute the column with 20 mL of hexane. Collect the first 20 mL of hexane solution eluting from the column. This first fraction contains all the target compounds mentioned earlier except tributyl phosphate, β-HCH, dieldrin. Elute the column with a solution of hexane : acetone (90 : 10). Collect the next 20 mL of solvent eluting from the column. This second fraction contains tributyl phosphate, β-HCH, dieldrin. Concentrate each fraction up to about 5 mL using a KD concentrator. Add the internal standard, then concentrate further to 1 mL by evaporating the solution under a stream of nitrogen. Finally, introduce the concentrated extract into the GC/MS for analysis (note 5).

b) Sediment samples

Load the concentrated solution obtained after KD concentration in part III.2.1.5 A (b) (note 6) on to a silica gel column. Elute the column with 20 mL of hexane. Collect the first 20 mL of hexane solution eluting from the column (first fraction). Elute the column with 50 mL of hexane : acetone (99 :1) (second fraction). Elute the column with 40 mL of hexane : acetone (90 :10) (third fraction). Concentrate the three fractions using a KD concentrator to a final volume of 1 mL. Load the concentrated third fraction onto an activated charcoal column, and elute with 20 mL of hexane : acetone (70 : 30). Concentrate the eluted solution with a KD concentrator up to a final volume of about 1 mL. Add internal standard to the three fractions, then concentrate further to exactly 1 mL by evaporating the solution under a stream of nitrogen. Finally, introduce the concentrated extract into the GC/MS for analysis (note 5).

C. Preparation of blank samples

Add surrogate into purified water (the same amount as added to samples (25 mL for sediment analysis)) and then treat the blank sample in the same manner as real samples.

D. Preparation of standard solutions

Prepare standard solutions for a calibration curve by mixing target compounds, surrogates, and internal standards in hexane. Surrogates and internal standards should be added in the same amount as that added to samples. Target compounds should be added in amounts that, when the standard solution is injected into the GC/MS will produce an MS response near the high end of the linear region of the calibration curve. (if necessary anticipate this concentration based on instrumental detection limit). Prepare at least five concentrations.

E. Analysis

a) Analytical condition of GC/MS

(1) **GC**

- column : fused silica capillary column (30 m x 0.25 mm i.d., 0.25μm)
- liquid phase : methyl silicone or 5% phenylmethyl silicone
- column temperature : 50 °C (1 min) - 6 °C/min - 100 °C - 15 °C/min - 280 °C
- injection temperature : 230 °C
- injection method : splitless method (1 min for purge-off time), 1μL injection
- carrier gas : He, average linear velocity : 40 cm/sec

- inlet temperature : 280 °C

(2) **MS**

- ionisation method : EI
- ionisation current : 300 μA
- ionisation voltage : 70 eV
- ion source temperature : 220 °C
- detection mode : SIM

(3) **Monitor ions (( ) is m/z of confirmatory ion)**

- Target compounds

  HCB : 284 (286), dieldrin : 263 (277), p,p'-DDE : 246 (318), p,p'-DDD : 165 (235), p,p'-DDT : 165 (235), trans-chlordane : 373 (375), cis-chlordane : 373 (375), trans-nonachlor : 407 (409), cis-nonachlor : 407 (409), α-HCH : 181 (219), β-HCH : 181 (219), o-terphenyl : 230, m-terphenyl : 230, p-terphenyl : 230, BHT : 205, benzo(a)pyrene : 252, tributylphosphate : 99 (151, 211)

- Surrogate compounds

  HCB-$^{13}C_6$: 290 (292), p-terphenyl-d_{14} : 244, benzo(a)pyrene-d_{12} : 264

- Internal standard compounds

  naphthalene-d_8 : 136, fluoranthene-d_{10} : 212, perylene-d_{12} : 264

F. Calibration curves

Make calibration curves for HCB, terphenyls and benzo(a)pyrene by the internal standard method using their stable isotope labelled compounds (HCB-$^{13}C_6$, p-terpneyl-d_{14}, and benzo(a)pyrene-d_{12}). For other compounds, make calibration curves by the internal standard method using internal standards (naphthalene-d_8, fluoranthene-d_{10}, perylene-d_{12}) whose retention times are close to the retention times of target compounds.

G. Determination

Determine by the isotope dilution method when adding HCB-$^{13}C_6$, p-terphenyl-d_4, and benzo(a)pyrene-d_{12} into samples. For other compounds, determine by the internal standard method.

III.2.1.6 Notes

(1) Cover and protect from light during analysis because benzo(a)pyrene is easily degraded by light. In general, protect all standard solutions etc. from light.

(2) Add surrogates to the samples in amounts similar to the anticipated amounts of target compounds in the samples.

(3) Conduct silica gel clean-up before adding internal standards if samples contain compounds that interfere with GC/MS analysis.

(4) Determine the elution pattern and recovery rate of each of the compounds during column chromatography before actual analysis, and change conditions if there are any problems.

(5) May analyse combined fractions if there is no interference with GC/MS analysis. In this case, after collecting all fractions, concentrate, then add internal standards for GC/MS analysis.

(6) When the extracts before clean-up have large amount of precipitate, conduct sample clean-up using silica gel column for water analysis as a preliminary clean-up method.

(7) Elemental sulfur interferes with GC/MS analysis, conduct a reduction copper treatment.

(8) Reduction copper treatment : Add 2 g of reduction copper into the eluate, shake vigorously to mix, then collect the eluate.

III.2.2 Analytical method for volatile compounds in ambient air using canisters

III.2.2.1 Target compounds

Acrylonitrile, vinyl chloride monomer, chloroform, 1,2-dichloroethane, dichloromethane, tetrachloroethylene, trichloroethylene, benzene, 1,3-butadiene

III.2.2.2 Outline of analytical methods

Collect the air samples under conditions of constant flow using sampling containers made of stainless steel. Analyse a specified fraction of the sample by capillary GC/MS.

III.2.2.3 Detection limit and determination limit

The optimum (target) determination limits of the above mentioned target compounds are shown in **Table III-2-1**. Follow all analytical procedures from sample concentration to GC/MS measurement using a sample container which is filled with a humidified mixture of standard gas. This mixture should have a concentration close to the estimated minimum determination limit obtained at the time of making calibration curve. Calculate the concentration of the humidified

mixture of standard gas. Calculate the detection limit and determination limit values for each target compound using the following equations, and values obtained for the standard deviation (s) from more than five standard gas samples. However, in the case of the samples which have operating blanks, measure the operating blank, and then choose whichever of the humidified mixture standard gas or operating blank has the larger standard deviation value. If the minimum determination limit value obtained is higher than required to satisfy the survey's purpose or the optimum determination limit given **Table III-2-1**, adjust to make less than purpose value checking apparatus and instruments. Perform measurements more than once to optimise instrument analysis conditions.

detection limit value = 3s ($\mu g/m^3$)

determination limit value = 10s ($\mu g/m^3$)

Table III-2-1 Optimum determination limits of target compounds

Target compounds	Optimum determination limit ($\mu g/m^3$)
acrylonitrile	0.01
vinyl chloride monomer	0.1
chloroform	0.04
1,2-dichloroethane	0.04
dichloromethane	2.0
tetrachloroethylene	20
trichloroethylene	20
1,3-butadiene	0.004
benzene	0.3

III.2.2.4 Reagents and apparatus

A. Reagents

- Zerogas : Use high purity nitrogen or purified air with concentrations of target compounds lower than optimum (target) determination limit value. Check the concentration of the target compounds before the use. It is important that the zerogas does not contain organic compounds, and it is desirable that for all compounds except target compounds the total hydrocarbon content is less than 0.01 ppm, carbon monoxide less than 0.05 ppm, carbon dioxide less than 0.3 ppm, humidity less than 2 ppm, the dew point less than -70 ◦C, and purity more than 99.999%.
- Humidified zerogas : Prepare humidified zerogas by bubbling zerogas through water (note 1) (relative humidity at 25 ◦C is about 60 - 70 %). Or prepare humidified zerogas by passing zerogas into an evacuated container and inject water by syringe (about 100 μL for 6 L container : relative humidity at 25 ◦C increasing pressure is about 50 %). In either case, be

careful about contamination when humidifying.

- Standard reagent : special grade reagents of purity more than 98 % or similar grade.
- Standard compounds : special grade reagents of purity more than 98 % or similar grade. When target compounds are gasses, permeation tubes can be used.
- Stock standard gas (1 μg/mL) : Use commercially available standard gases in cylinders. Convert gas concentration from ppm (μL/L) on the commercial gas cylinder into weight / volume concentration (μg/L) using the equation 273xM(22.4x(273+t)) (M : molecular weight, t : temperature). The concentration of stock standard gas (1 μg/mL) is a rough standard - it can be changed for each target compound, bearing in mind the compound's sensitivity or atmospheric concentration. (note 2)
- Humidified standard gas mixture (0 - 0.1 ng/mL) : Use thoroughly washed, non-contaminated sampling containers, and prepare the humidified standard gas mixture at five levels of concentration between 0 - 0.1 ng/mL by diluting the stock standard gas (1 μg/mL) with humidified zerogas (using pressure dilution, capacity ratio mixture, or air flow ratio mixture methods to meet the determination range of each target compound). Prepare humidified standard gas mixture by pressurising (about 200 kPa). (note 3)
- Internal standard compounds : toluene-d_8, fluorobenzene, chlrorobenzene-d_5 etc.
- Internal standard stock gas (1 μg/mL) : Use commercial standard gases.
- Humidified internal standard gas (0.01 ng/mL) : dilute internal standard stock gas with humidified zerogas. Use a different container. Dilute with sufficient gas to meet the optimised concentrations as it is used. (note 4)

B. Apparatus and instruments

a) Sampling equipment

Collect samples using sample containers which are under reduced pressure (less than 13 Pa (about 0.1 mmHg)). Draw the samples into the container under conditions of constant flow using either a mechanical mass flow controller or a thermal mass flow controller. These instruments' have different functions which depend on the sampling methods. There are essentially two methods : subatomospheric pressure sampling method, where sampling is terminated while the container is still at less than atmospheric pressure, and the pressurized sampling method, where sample is pumped into the container to above atmospheric pressure. Pressures of up to 200 kPa (about 1500 mmHg) are attained.

Those parts of the sampling equipment which touch the target compounds, or the tubing, are made of stainless steel inactivated by electrolytic polishing or aluminium coating or an equivalent process. Where non-metallic material is used, it is necessary to check that there is no interaction

with target compounds beforehand. Such materials, except fluorine resin or polyimide, should be avoided where possible.

Prior to sampling, wash the apparatus thoroughly to avoid contamination. When sampling, check for leak after setting up the equipment, wash the sampling apparatus and replace the internal atmosphere by sample air. Endeavour to minimise all risk of contamination and adsorption.

(1) Subatomospheric pressure sampling apparatus

This comprises a filter, mass flow controller, valve, sample container and a pressure meter with which one can check the pressure inside the sample container. The pressure at the end of sampling should be within the range at which the mass flow controller can maintain a constant flow, and this pressure is generally about 80 kPa (80 % of atmospheric pressure).

(2) Pressurized sampling apparatus

This comprises a filter, pump, mass flow controller, valve, pressure meter sample container and a pressure meter with which one can check the pressure inside the sample container. The pressure at the end of sampling is about 200 kPa (about 1500 mmHg).

(i) Sample container

The container capacity should be between 3 - 15 L. Use stainless steel containers whose interior surfaces have been inactivated by electrolytic polishing or aluminium coating. If such stainless steel containers are not available, use any other container made of material of similar or better quality. Use containers from which target analyte recovery rates and storage quality have been confirmed. Ensure the containers do not leak, and can withstand pressurisation to about 300 kPa (about 2200 mmHg), and depressurisation to about 13 Pa (about 0.1 mmHg).

(ii) Mass flow controller

Use a stainless steel mass flow controller whose interior surfaces have been inactivated by electrolytic polishing or aluminium coating. If such stainless mass flow controllers are not available, use any other flow controller made of material of similar or better quality. Use a mass flow controller which can control the flow rate to within 2 - 50 mL/min, control the accuracy of the flow rate at more than 20 kPa (about 150 mmHg), and can control pressure differences to within ± 10 % of the set flow rate. Ensure the flow controller does not leak, and can withstand pressurisation to about 300 kPa (about 2200 mmHg), and depressurisation to about 13 Pa (about 0.1 mmHg). Connection parts are made of stainless or aluminium coated by oxidation or similar or better quality.

(iii) Pump : for analysis of harmful air pollution compounds

(iv) Valve : for analysis of harmful air pollution compounds

(v) Filter : made of stainless, and mesh size is less than 7 μm, generally around 2 μm.

(vi) Pressure meter : for analysis of harmful air pollution compounds

b) Sample introducing apparatus

(1) Concentrator (adsorption concentrator tube or low temperature concentrator tube)

Use adsorption concentrator tubes when one wants to concentrate target compounds by means of adsorption onto a sorbent material. Adsorption concentrator tubes are generally glass tubes (1 - 3 mm i.d.), glass lined stainless tubes, or stainless steel tubes packed with porous polymer beads or carbon adsorbent (either singly or combined). Both ends of the tube are usually packed with deactivated quartz wool. Adsorption concentrator tubes can be heated at 180 °C for desorption. However the maximum heating temperature depends on the adsorbent.

Low temperature concentrator tubes are used when one wants to concentrate target compounds by means of adsorption onto a sorbent material at low temperatures. Low temperature concentrator tubes can be heated more than 90 ◦C for desorption. Low temperature concentrator tube are generally glass tubes (1 - 6 mm i.d.), glass lined stainless tubes or stainless tubes packed with deactivated glass beads (250 - 500 μm diameter), quartz beads (250 - 500 μm diameter), quartz wool or deactivated diatomaceous earth (250 - 500 μm diameter) etc. (note 5)

(2) Cryo focus

The cryo-focus is a trap attached to the front of the capillary column. The cryo-focus is used to narrow the band of target compounds entering the column. Generally, the cryo-focus is a fused silica or deactivated stainless steel chambered tube (0.3 - 0.6 mm i.d.), the temperature of which can be controlled at below -100 °C, and yet heated to more than 80 °C instantly.

(3) Dehumidifier

The dehumidifier, as its name suggests, dehumidifies the samples before concentration. Dehumidification is achieved using high molecular membranes which selectively transport water (e.g. Nafion Dryer - Perma Pure Products), dry purge methods, or other methods which can exclude volatile compounds selectively from water (e.g. by purge & trap theory, etc). Polar compounds such as acrylonitrile shouldn't be effected by the dehumidifier.

III.2.2.5 Experimental

A. Sampling

Sample by either the subatomospheric pressure sampling method or the pressurized sampling method. At the same time, make a travel blank and for 10% of samples, collect two samples for analysis (note 7). For example, when 10 samples are taken, prepare at least one travel blank, and for at least one sample collect two samples for analysis. If samples are taken by the subatomospheric pressure sampling method, record the pressure before and after pressurising samples, and calculate dilution rate after pressurising.

B. Concentration of samples

If the samples were collected by the subatomospheric pressure sampling method, pressurise the sample container with humidified zerogas. Connect the sample containers to the sample introduction apparatus, and concentrate the samples at a constant flow rate. If the samples were collected by the pressurized sampling method, connect the sample containers directly to the sample introduction apparatus, and concentrate the samples at a constant flow rate. Control the flow rate by using the mass flow controller, and end the concentration procedure after a prescribed period of time has elapsed. Determine the amount of samples which has been concentrated by using the concentration of target compounds and the sensitivity of the analytical instruments. At this time concentrate a known amount of humidified internal standard gas. The amount of gas used should be the same as the amount gas used when making calibration curves.

Desorb target compounds by heating the concentrator (e.g. at 180 °C for the adsorption concentrator, at 90 °C for the low temperature concentrator), and re-concentrate on the cryo-focus. However, if there is leakage (a pressure difference of more than ±10 kPa between the end of sampling and analysis inside the container), stop analysing.

C. Preparation of blank samples

a) Operational blank

After washing, conduct the whole procedure from sample concentration to GC/MS measurement using sample containers pressurised up to 200 kPa (about 1500 mmHg) with humidified zerogas, and gain operational blank value. (note 8)

b) Travel blank

Conduct the whole procedure from sample concentration to GC/MS measurement using sample containers for travel blank experiments, and measure target compound quantity in the

concentrated samples. (note 9)

D. Measurement

Measurement is done by SIM method or the scanning method using the following GC/MS conditions. Monitor ions are shown in **Table III-2-2**. When target compounds are detected, obtain the peak area or height of the primary ion of the target compounds and internal standards, and calculate the weight of the target compounds from their respective calibration curves.

An example of GC/MS conditions

- column : methylsilicone capillary column, 0.25 mm i.d., length 60 m, film thickness 0.25 μm
- column temperature : 40 °C (5 min) - 4 °C/min - 140 °C
- interface temperature : 220 °C
- carrier gas : He, 1 - 3 mL/min
- ion source temperature : 200 °C

Table III-2-2 Monitor ions

Compounds	Primary ions	Secondary ions
Acrylonitrile	52	53
Vinyl chloride monomer	62	64
Chloroform	83	85
1,2-Dichloromethane	62	64
Dichloromethane	84	86, 49
Tetrachloroethylene	166	164, 129
Trichloroethylene	130	132, 95
Benzene	54	53
1,3-Butadiene	78	77
Toluene-d_8	98	-
Fluorobenzene	96	-
Chlorobenzene-d_5	117	-

E. Calibration curves

(a) Connect a container of minimum concentration humidified standard gas to the sample introduction apparatus, and concentrate 100 mL of that mixture in the concentrator. Then add 100 mL of humidified internal standard gas into the concentrator and concentrate together. Thereafter, operate all analytical procedures from sample concentration to GC/MS analysis to record chromatograms of each target compounds. Repeat the same procedure using a container of humidified standard gas mixture of a higher concentration. (note 10)

(b) Choose an injection volume which will produce concentrations in the middle of the GC/MS calibration curve. Obtain this information from the standard gas mixture calibration curves obtained from procedure (a) above. Obtain the ratio of the peak intensities of the primary ion and secondary ion of each target compound. (note 11)

(c) Obtain the ratio of intensities of the peak intensities of the primary ion and secondary ion of each target compound at each concentration, and check if they coincide with the intensity ratio of each target compound obtained using procedure (b) above. (note 12)

d) Calculate the ratio of peak intensities of the primary ion of each target compound and the internal standard, and make calibration curves from the ratio of peak intensity and quantity of each target compound.

F. Determination and calculation

Calculate the concentration of each target compound in the air from the results obtained during analysis of samples and travel blanks by using the following formula.

$$C = \frac{n \times (As - At)}{v \times 293/(273 + t)} \times \frac{Pa}{101.3}$$

C : concentration of the target compound in the air at 20 °C (μg/m^3)

n : dilution magnification (for subatomospheric pressure sampling method. n=1 for pressurized sampling)

As: weight of each target compounds in the concentrated samples (ng)

At: travel blank of each target compounds (ng)

v : concentrated amount of samples for analysis. use operational blank value if it is the same.

t : temperature at sample analysis

Pa: atmosphere pressure at sample analysis

III.2.2.6 Notes

(1) Be careful to use commercial mineral water because salts can be deposited at connections and on the inside of the sample containers. When operational blank values are not stable, the use of purified water (water which has been boiled to half of its original volume on a gas burner, and cooled evaporative cooling in a stream of helium gas) is recommended.

(2) To prepare stock standard gas, accurately weigh around 100 mg of a target compound standard (either individually or as part of a mixture) and inject the standard material into a 1 L glass vacuum bottle (whose internal volume is accurately measured by replacing with high quality nitrogen and back to atmospheric pressure). Heat the vacuum bottle to more than 60 °C to evaporate the standard compounds. Dilute the thoroughly volatilised, thoroughly mixed gas mixture 100 times in a second vacuum bottle to make the standard stock gas. If the target compound standard is a gas contained in a cylinder, the 100 mg of target compound can be measured using a gas-tight gas syringe. In this case, the amount of material injected is determined using the following formula : v (mL) = 100x(22.4x(273+t)/273M (M : molecular weight, t : atmospheric temperature), If the target compound standard is a liquid, the 100 mg of target compound can be measured using a micro syringe. In this case, the amount of material injected is determined using the following formula : v (μL) = 100/ρ (ρ: specific gravity or density).

(3) Pressure dilution is a kind of capacity ratio mixing. The dilution ratio is calculated from pressure increments. In the case of dilution using humidified zerogas prepared in the container, be careful that the relative humidity does not become lower because of dilution.

(4) To prepare internal standard stock gas, accurately weigh around 100 mg of the standard and inject the standard material into a 1 L glass vacuum bottle (whose internal volume is accurately measured by replacing with high quality nitrogen and back to atmospheric pressure). Heat the vacuum bottle to more than 60 °C to evaporate the standard compounds. Dilute the thoroughly volatilised, thoroughly mixed gas 100 times in a second vacuum bottle to make the standard stock gas. If the internal standard is a gas contained in a cylinder, the 100 mg of the standard can be measured using a gas-tight gas syringe. In this case, the amount of material injected is determined using the following formula : v (mL) = 100x(22.4x(273+t)/273M (M : molecular weight, t : atmospheric temperature). If the internal standard is a liquid, the 100 mg of the standard can be measured using a micro syringe. In this case, the amount of material injected is determined using the following formula : v (μL) = 100/ρ (ρ: specific gravity or density).

(5) Liquid nitrogen (b.p.=-196 °C) and liquid oxygen (b.p.=-183 °C) can be used as the refrigerant for low temperature concentration at concentrator. However, liquid nitrogen

may clog the tubing because of oxygen condensation from samples. Also, check the tubing regularly, because tubing clogging may be caused by water or carbon dioxide etc. at the time of low temperature concentration.

(6) Be careful about trap tube because water or carbon dioxide may cause clog tubing. Use of the trap tubing may omitted if sample recovery from the concentrator is rapid, and the component peaks which come out at the beginning show enough shape to be determined.

(7) Confirm that the difference in determined concentration of target compounds between the two analyses is less than 30 %, and more than determination limit value. If the difference is more than 30 %, as a matter of principle record the analyses as 'not determined', check the reason, and repeat the sampling. Perform this operation before sample measurement. If the value when converted to atmospheric concentration is over target determination limit value, repeat the measurement after re-washing and machine adjustment, to obtain operational blank values of low enough concentration, and measure samples.

(8) If a value of a target compound in travel blank is the same (or smaller) as an operational blank value, calculate concentration by subtracting the operational blank value from a measurement value of a sample.

(9) If a travel blank value is larger than an operational value; confirm that (a) the determination limit value (10s : converted into atmospheric concentration) calculated from standard deviation (s) of travel blank values from more than three samples is smaller than the optimum determination limit in Table III-2-1, or (b) even if the determination limit value calculated from the travel blank values is larger than the optimum determination limit, a measured value for the sample is larger than the determination limit value from travel blank values. If either of these is satisfied, calculate concentration by subtracting the travel blank value from the sample measurement value. However, if none of these, as a matter of principle treat as 'not determined'. In this case repeat sampling after removing causes of contamination.

(10) In the case which it is confirmed that recovery from container is from 80 to 120 %, standard stock gas can be injected into concentrator directly using a gas syringe.

(11) This operation is in order to confirm target compounds and use every calibration making.

(12) If the intensity ratio of any of the target compounds exceeds 90 - 110 % of the value which is calculated from calibration curves, repeat the analysis of the concentration of the standard gas.

III.2.3 Analysis of dioxins in sediments and biological samples

III.2.3.1 Target compounds

Dioxins (polychlorinated dibenzo-*p*-dioxin (PCDDs) and polychlorinated dibenzofuran (PCDFs))

III.2.3.2 Outline of analytical methods

Extract the dioxins from sediments and biological samples, treat the extracts with sulfuric acid and then clean-up the extracts by column chromatography. Finally, determine PCDD and PCDF concentrations in the extracts by high resolution gas chromatography / mass spectrometry (HRGC/HRMS).

III.2.3.3 Detection limit and determination limit

Target (optimum) determination limits are shown in **Table III-2-3**.

Table III-2-3 Target determination limits

Compounds	Target determination limits
Tetra CDD	0.1 pg/g
Tetra CDF	0.1 pg/g
Penta CDD	0.1 pg/g
Penta CDF	0.1 pg/g
Hexa CDD	0.2 pg/g
Hexa CDF	0.2 pg/g
Hepta CDD	0.2 pg/g
Hepta CDF	0.2 pg/g
Octa CDD	0.5 pg/g
Octa CDF	0.5 pg/g

Sediments : per dry weight
Living things : per wet weight

Measure 1 μL of standard solution of a concentration near the lowest concentration which could be determined at the time of making calibration curves (the standard concentration should be around detection limit). Determine the value of this solution (A pg). Calculate the concentration of samples from the concentration formula. Repeat this measurement five times and calculate standard deviation(s) of the measurements. Obtain detection limits and limits of determination of dioxins from the following formula.

Detection limits = 3s (ng/g) (sediment : per dry weight, living things : per wet weight)

Determination limits = 10s (ng/g) (sediment : per dry weight, living things : per wet weight)

If the value for the operational blank is not zero, measure operational blank in the same way, calculate the standard deviation, and then calculate detection limits and determination limits using whichever standard deviation from the standard solution or the operational blank is the larger. If the determination limits are larger than the target determination limits, check the apparatus and instruments, and adjust conditions to make the determination limits less than the target values.

III.2.3.4 Reagents and apparatus

A. Reagents

- methanol, acetone, hexane, toluene, dichloromethane : pesticide residue analysis grade or PCB residue analysis grade
- nonane, decane, isooctane : pesticide residue analysis grade
- hexane washed water : distilled water thoroughly washed with hexane
- sulfuric acid, hydrochloric acid : reagent special grade or higher grade
- anhydrous sodium sulfate : pesticide residue analysis grade or PCB residue analysis grade
- potassium hydroxide, sodium hydroxide : reagent special grade
- silica gel : wash silica gel for column chromatography with methanol, place in a beaker to a maximum depth (thickness) of 10 mm thickness, dry the layer of silica gel at 130 °C for about 18 hours, then cool in a desiccator over 30 minutes.
- basic alumina : aluminum oxide 90 (activity 1) 70 - 230 mesh (Merck)
- copper powder : Merck, washed with hexane beforehand
- silica gel with activated charcoal : for dioxin analysis
- target compounds : target compounds of dioxins are shown in **Table III-2-4**. Standard mixtures are commercially available.
- internal standard : internal standard for the use is shown in **Table III-2-5**. Standard mixtures are commercially available.

Table III-2-4 Dioxins for analytical target

number of chlorines	dioxins	dibenzofurans
tetra chlorinated	1,3,6,8/1,3,7,9-T_4CDD Isomer Pair 2,3,7,8-T_4CDD	1,3,6,8-T_4CDF 2,3,7,8-T_4CDF
penta chlorinated	1,2,3,7,8-P_5CDD	1,2,3,7,8-P_5CDF 2,3,4,7,8-P_5CDF
hexa chlorinated	1,2,3,4,7,8-H_6CDD 1,2,3,6,7,8- H_6CDD 1,2,3,7,8,9- H_6CDD	1,2,3,4,7,8- H_6CDF 1,2,3,6,7,8- H_6CDF 1,2,3,7,8,9- H_6CDF 2,3,4,7,8,9- H_6CDF
hepta chlorinated	1,2,3,4,6,7,8-H_7CDD	1,2,3,4,6,7,8-H_7CDF 1,2,3,4,7,8,9-H_7CDF
octa chlorinated	1,2,3,4,6,7,8,9-O_8CDD	1,2,3,4,6,7,8,9-O_8CDF

Table III-2-5 Internal standards

Number of chlorines	Internal standards for dioxins	Target dioxins	Internal standards for dibenzofurans	Target dibenzofurans
tetra chlorinated	$^{13}C_{12}$2,3,7,8-T_4CDD	for determination of T_4CDD	$^{13}C_{12}$2,3,7,8-T_4CDF	for determination of T_4CDF
penta chlorinated	$^{13}C_{12}$1,2,3,7,8-P_5CDD	for determination of P_5CDD	$^{13}C_{12}$1,2,3,7,8-P_5CDF	for determination of P_5CDF
hexa chlorinated	$^{13}C_{12}$1,2,3,6,7,8-H_6CDD	for determination of H_6CDD	$^{13}C_{12}$1,2,3,6,7,8-H_6CDF	for determination of H_6CDF
hepta chlorinated	$^{13}C_{12}$1,2,3,4,6,7,8-H_7CDD	for determination of H_7CDD	$^{13}C_{12}$1,2,3,4,6,7,8-H_7CDF	for determination of H_7CDF
octa chlorinated	$^{13}C_{12}$1,2,3,4,6,7,8,9-O_8CDD	for determination of O_8CDD	$^{13}C_{12}$1,2,3,4,6,7,8,9-O_8CDF	for determination of O_8CDF

B. Apparatus

a) Extraction apparatus

- glassware : separating funnels, Erlenmeyer flasks with ground-glass joints and stoppers, round bottom flasks, chromatography columns, test tubes for concentration. All glassware should be washed with hexane beforehand.
- rotary evaporator
- mechanical shaker

b) Apparatus for clean-up

- silica gel chromatography : chromatography column (i.d., 10 mm; length, 300 mm) packed with 3 g of activated silica gel. Mix the silica gel with sufficient hexane to form a free flowing slurry and load into column. Add a 10 mm layer of anhydrous sodium sulfate onto the top of the silica gel. Wash the packed material thoroughly with hexane.
- alumina column chromatography: chromatography column (i.d., 10 mm; length, 300 mm)

packed with 15 g of basic alumina. Mix the alumina with sufficient hexane to form a free flowing slurry and load into column. Add a 10 mm layer of anhydrous sodium sulfate onto the top of the alumina. Wash the packed material thoroughly with hexane.

- activated charcoal column chromatography: chromatography column (i.d., 10 mm; length, 300 mm). Packed the column first with a 10 mm layer of anhydrous sodium sulfate, then with 1 g of a mixture of silica gel and activated charcoal. Finally, add 10 mm layer of anhydrous sodium sulfate onto the top of the silica gel. Wash the packed material thoroughly with hexane.

c) GC/MS

- GC : GC for capillary column
- MS : double-focusing type, more than 10,000 resolution

III.2.3.5 Experimental

A. Sample preparation

a) Sediment samples

Weigh 50 - 100 g of air-dried mud (note 2) into a round bottomed flask. Add 0.2 mL of a hexane solution containing 0.01 μg/mL each of 10 kinds of internal standard (note 3). Then add 200 mL of acetone-hexane solution (1:1 v/v) and 2 g of copper powder, and shake for one hour. Filter the extracts, wash the flask and the residue with 100 mL of acetone-hexane solution (1:1 v/v), combine the filtrates, and place into a separating funnel. Add 200 mL of purified water, and shake for 10 minutes. After settling, discard the aqueous phase, add 100 mL of 2 % sodium chloride solution, then shake. After settling, discard the aqueous phase, add 100 mL of 2 % sodium chloride solution, then shake for 10 minutes. After settling discard the aqueous phase, dehydrate hexane layer by passing through the funnel packed with 20 g of anhydrous sodium sulfate, and concentrate by rotary evaporation to about 20 mL using a bath temperature of less than 40 °C. Add 20 mL of 1 M potassium hydroxide solution into the concentrated organic phase, shake, settle, and make up the hexane layer a certain volume (L : mL). Separate about the half amount (s : mL) as a sample for clean-up (note 4).

b) Biological samples

Weigh 100 g of homogenised samples into a round bottomed flask. Add 0.2 mL of a hexane solution containing 0.01 μg/mL each of 10 kinds of internal standard (note 3). Then add 200 mL of

2M potassium hydroxide and 150 mL of methanol, shake or stir, and leave the mixture overnight at room temperature. Transfer the mixture to a separating funnel, add 150 mL of hexane, then shake for 10 minutes. After settling, separate the hexane layer. Wash the aqueous phase with a further 100 mL hexane. After settling, separate the hexane layer. Repeat this procedure twice. Combine the hexane extracts, add 200 mL of 2 % sodium chloride solution, then shake gently (swirl or rotate the mixture). After settling, discard the aqueous phase. Add 100 mL of 2 % sodium chloride to the organic phase (hexane layer), and repeat the wash procedure. After settling discard the aqueous phase, dehydrate hexane layer by passing through the funnel packed with 20 g of anhydrous sodium sulfate, and concentrate by rotary evaporation to about 20 mL using a bath temperature of less than 40 °C. Make up a certain volume (L : mL). Separate about the half amount (s : mL) as a sample for clean-up (note 4).

B. Sample clean-up

(1) Sulfuric acid washing

Take a certain amount (s : mL) of the extracts into a separating funnel, add 50 - 150 mL of hexane and 10 mL of concentrated sulfuric acid, and shake gently. Allow the mixture to settle, then discard the sulfuric acid layer. Repeat this procedure until the sulfuric acid layer is colourless. Wash the hexane layer 3 or 4 times with 10 mL of hexane-washed water, then add 10 mL of 5 % sodium bicarbonate solution, and shake gently. After settling discard the aqueous phase, dehydrate hexane layer by passing through the funnel packed with 10 g of anhydrous sodium sulfate, and concentrate the samples for silica gel column chromatography by rotary evaporation to about 5 mL using a bath temperature of less than 40 °C.

(2) Silica gel column chromatography

Load samples from (1) onto the column, and elute slowly (eluate drip speed of 1 drop per second) with 200 mL of hexane. Concentrate the eluate samples for alumina column chromatography with a rotary evaporator to about 5 mL using a bath temperature of less than 40 °C.

(3) Alumina column chromatography (note 5)

Load sample from (2) onto the column, and elute slowly (eluate drip speed of 1 drop per second) with 30 mL of dichloromethane-hexane solution (2 : 98 v/v) (first fraction). Then elute slowly (eluate drip speed of 1 drop per second) with 200 mL of dichloromethane-hexane solution (6 : 4 v/v) (second fraction). Place the second fraction into a round bottomed flask, and concentrate the samples for activated charcoal column chromatography by rotary evaporation to about 0.5 mL

using a bath temperature of less than 40 °C.

(4) Activated charcoal column chromatography

Wash activated charcoal column with toluene, and replace by hexane thoroughly. Load the concentrate from (3) onto the column, and elute slowly (eluate drip speed of 1 drop per second) with 100 mL of dichloromethane-hexane solution (1:3 v/v). This fraction contains PCBs. Then elute dioxins with 200 mL of toluene. Concentrate the toluene layer by rotary evaporation to about 5 mL using a bath temperature of less than 40 °C. Concentrate further to 1 mL by blowing nitrogen over the surface of the solution, add 0.5 mL of decane and concentrate up to 100 μL (E : mL) for GC/MS analysis (note 6).

C. Preparation of blank samples

Prepare blank samples using the same operating procedures as used for samples (note 7). Also analyse some samples twice for quality control (note 8).

D. Preparation of standard solutions

- standard solutions (50ng/mL) : dilute commercial standard mixture solution (50μg/mL) 1,000 times with toluene
- internal standard solutions (50ng/mL) : dilute commercial standard mixture solution (50μg/mL) 1,000 times with toluene

E. Analysis

a) GC/MS conditions

(1) **GC**

- column

(a) fusued silica SP-2331 capillary column, 0.25 mm i.d. x 30 m, 0.2 μm

(b) fused silica DB-17 capillary column, 0.25 mm i.d. x 30 m, 0.15 μm

- column temperature

(a) 150 °C (1 min) - 15 °C/min - 200 °C (5 min) - 3 °C/min - 250 °C (30 min)

(b) 150 °C (1 min) - 15 °C/min - 270 °C (30 min)

- injection port temperature : 260 °C
- injection method : splitless (90s for purge-off time)

(2) **MS**

- resolution : 7000 - 10000

- ionisation method : EI
- ionisation voltage : 30 eV
- ionisation current : 500 μA
- ion source temperature : 260 °C
- detection method : SIM

Introduce the standard for mass adjustment (PFK) into the MS, and adjust the mass pattern, resolution (more than 10000, 10 % Valley), etc. by using the mass adjustment program to meet the analysis purpose. **Table III-2-6** shows monitor ions.

Table III-2-6 List of monitor ions

	Chlorinated substituent	M^+	$(M+2)^+$	$(M+4)^+$
Target compounds	T_4CDDs	319.8965	321.8936	
	P_5CDDs	353.8576	355.8546	357.8516
	H_6CDDs		389.8157	391.8127
	H_7CDDs		423.7766	425.7737
	O_8CDDs		457.7377	459.7348
	T_4CDFs	303.9016	305.8987	
	P_5CDFs		339.8597	341.8567
	H_6CDFs		373.8207	375.8178
	H_7CDFs		407.7818	409.7789
	O_8CDFs		441.7428	443.7399
Internal standards	$^{13}C_{12}T_4CDD$	331.9368	333.9339	
	$^{13}C_{12}P_5CDD$	365.8978	367.8949	369.8919
	$^{13}C_{12}H_6CDD$		401.8559	403.8530
	$^{13}C_{12}H_7CDD$		435.8169	437.8140
	$^{13}C_{12}O_8CDD$		469.7779	471.7750
	$^{13}C_{12}T_4CDF$	315.9419	317.9389	
	$^{13}C_{12}P_5CDF$		351.9000	353.8970
	$^{13}C_{12}H_6CDF$		385.8610	387.8580
	$^{13}C_{12}H_7CDF$		419.8220	421.8191
	$^{13}C_{12}O_8CDF$		453.7830	455.7801

F. Calibration curves

Prepare standard solutions of each chlorinated compound. Make calibration curves using five points, between 0.5 ng/mL and 1 μg/mL (note 9), and include zero. Then add a known amount of internal standards such that the concentration of the internal standard in the standard solutions being used to make the calibration curves is 0.01 μg/mL. Inject 1 μL of standard solution into the GC/MS, and measure. Obtain peak intensity ratio between determinative and confirmative ions of standard solutions which are in the mid-range of the calibration curves for each compound. Check if the peak intensity ratio between determinative and confirmative ions of other concentrations

coincide with those calculated for the mid-range concentration. Obtain peak intensities of each compound and internal standard, and make calibration curves against injection volumes.

G. Determination and calculation

Inject 1 - 2 μL of samples into the GC/MS system. Obtain peak intensities of the determinative ions of each chlorinated compound and the internal standards (note 10). Calculate the weight (As, pg) of each of the chlorinated compounds in the test sample injected from the calibration curves (note 11, 12). Calculate dioxin concentrations in the samples using the following formula.

$$C = \frac{As \times E \times L}{v \times s \times m}$$

where, C is the concentration of each of the chlorinated compounds in sample (ng/g) (sediment - ng/g based on dry weight, living things - ng/g based on wet weight)

As : weight of each chlorinated compound in the sample solution injected into the GC/MS (pg)

E : volume of sample solution for GC/MS analysis

v : volume injected into the GC/MS (μL)

L : original volume of extracts (mL)

s : amount of original extract separated for clean-up (mL)

m : amount of sample (g)

Calculate 2,3,7,8-T_4CDD conversion concentration (TEQ) by multiplying 2.3.7.8-T_4CDD Toxicity Equivalency Factor (TEF) by the measured concentration, and sum up them.

III.2.3.6 Notes

(1) Determine the most appropriate packing materials and solvents for column chromatography after performing fractionation experiments using standard compounds.

(2) Dry (air dry) samples trying not to evaporate dioxins from samples, or contaminate the samples with dioxins.

(3) It is desirable to add all internal standard compounds for determination, but add at least one internal standard for each chlorine numbers. All of these internal standards may at times interfere with analysis depending on the MS resolution set up, so consider and investigate

analytical conditions which don't produce interference. Use $^{13}C_{12}$-1,2,3,4-T_4CDD or $^{37}C1_4$-T_4CDD for confirmation of recovery rate of dioxins during sample preparation, $^{13}C_{12}$-1,2,3,4,6,7,8,9-O_8CDD for internal standard for determination. In addition, as well as $^{13}C_{12}$-1,3,6,8-T_4CDD, it is also necessary to check if dioxin separation on the chromatography column is done properly.

(4) Store original extracts for a while in case reanalysis of some of the samples is necessary.

(5) The activity of alumina varies depending on the age of the alumina (time of preservation after opening the product). 1,3,6,8-T_4CDD and 1,3,6,8-T_4CDF elute in the first fraction when the activity of the alumina goes down. Also O_8CDD may not elute at the defined hexane - dichloromethane ratio (4 : 6 v/v), and it is necessary to confirm this by fractionation tests.

(6) Conduct syringe spikes in order to obtain correct results. Use internal standards except clean-up spike compounds such as $^{13}C_{12}$1,2,3,4-T_4CDD or $^{13}C_{12}$-1,2,3,4,7,8,9-H_7CDF.

(7) If the values obtained from operational blank values exceed target determination limit values, repeat analysis after re-wash and check instruments to make operational blank values as low as possible, then analyse samples.

(8) Check if difference between the values of duplicate analysis whose concentrations are more than determination limit values, is less than 30 %. If the difference is above 30 %, treat as 'not determined' in principle, check the cause, and take samples again.

(9) This concentration range should include concentrations close to detection limit value and concentrations anticipated to be close to the high end of the linear dynamic range of GC/MS.

(10) Determine if the peak area ratio of monitor ions of SIM chromatograms is almost the same as standards, and within ±15 % of the natural isotope ratio (±25 % if around determination limit). In particular, determine 2,3,7,8- chlorinated isomers if SIM chromatogram peaks give good separation and retention times are almost the same as standards, and relative retention times against corresponding internal standards also agree with standards. For isomers which don't have standards, determine by reference to published articles etc.

(11) If recovery of clean-up spike is out of the range 50 % to 120 %, repeat clean-up from sample extracts and re-analyse.

(12) Conduct determination of 2,3,7,8- chlorinated isomers (17 isomers) using their corresponding standards. Determine other isomers using average of 2,3,7,8- chlorinated isomers of each chlorinated compound as standards.

III.2.4 Analytical methods for polycyclic aromatic hydrocarbons in water, sediment, biological organisms, and air

III.2.4.1 Target compounds

Benzo(a)pyrene(B(a)P),benzo(e)pyrene(B(e)P),benzo(b)fluoranthene(B(b)F),benzo(j)fluoranthene (B(j)F),benzo(k)fluoranthene(B(k)F),benzo(ghi)perylene(B(ghi)P),dibenz(a,h)anthracene(B(ah)A), 3-methylcholanthrene(MC)

III.2.4.2 Outline of analytical methods

In general, the analytical methods for polycyclic aromatic hydrocarbons in sediment, biological organisms, and air are similar to those used for water samples, i.e. after liquid-liquid extraction with hexane, concentrate the sample, conduct silica gel chromatography, and finally determine analyte concentrations by HPLC or GC/MS.

- For sediment and biological samples, first digest the sample using an alkaline (1M KOH-ethanol) solution, centrifuge, dilute with water, extract with hexane, and analyse as per water samples.
- For air samples, collect the samples by using an air sampler with a quartz filter paper, perform ultrasonic extraction with organic solvents, clean-up using SEP-PAK silica cartridges, then analyse as per water samples. Note : because GC/MS analysis cannot separate the three isomers of benzofluoranthene, treat these as one chemical when analysing by GC/MS.

III.2.4.3 Detection limits and determination limits

Detection and determination limits for the target chemicals (**III.2.4.1**) in water, sediment and biological samples are shown in **Table III-2-7**, detection limits for air samples in **Table III-2-8**.

Table III-2-7 Detection limits and determination limits of water, sediment and biological samples

units : μg/mL, μg/g

Sample	Sample amount		Analytical instruments	B(a)P	B(e)P	B(b)F	B(j)F	B(k)F	B(ghi)P	B(ah)A	MC
Water	1L	detection limits	HPLC	0.00005	0.00002	0.00006	0.0006	0.00004	0.00005	0.00004	0.00004
			GC/MS	0.00006	0.00001	0.00002	0.00002	0.00002	0.00004	0.00006	0.00004
		determination limits	HPLC	0.0002	0.00008	0.0002	0.002	0.0001	0.0002	0.0001	0.0001
			GC/MS	0.0002	0.00004	0.00007	0.00007	0.00007	0.0002	0.0002	0.0001
Sediment & living things	20 g	detection limits	HPLC	0.001	0.0006	0.0005	0.030	0.002	0.002	0.002	0.002
			GC/MS	0.003	0.0004	0.001	0.001	0.001	0.002	0.003	0.002

Table III-2-8 Detection limit of air samples

Compounds	Detection limits (pg)
B(a)P	26
B(e)P	14
BF	19
B(g,h,i)P	31
B(a,h)A	53
MC	24

III.2.4.4 Reagents and apparatus

A. Reagents

- pesticide residue analysis grade hexane, benzene, acetone, ethanol, anhydrous sodium sulfate
- HPLC grade methanol, distilled water
- KOH : special reagent grade
- target compounds : commercial standards
- p-terphenyl-d_{14}, perylene-d_{12} : Cambridge Isotope Laboratory
- 4% Na_2SO_4 solution : Add anhydrous sodium sulfate into purified water to make a 4 % w/v solution, and wash with hexane.
- silica gel : Activate silica gel S-1 at 130 °C for more than 8 hours.
- quartz filter paper : Watman Ltd. QM-A (8 x 10 inch). Heat filter papers in an electric furnace at 600 °C for four hours. Store the heat-treated filter papers in a desiccator.
- SEP-PAK silica cartridges : or similar products

B. Apparatus

- silica gel column : Pack 3 g of silica gel into a glass column (i.d. 1 cm, length 30 cm) with hexane, and lay about 2 cm height of anhydrous sodium sulfate on the top.
- rotary evaporator (with water bath) : for concentration of extracts
- KD concentrator : for concentration of extracts
- centrifuge : for separation-extraction of sediment and biological samples
- mechanical shaker : for liquid-liquid extraction
- aluminium foil : for covering columns etc during column chromatography
- glassware : separating funnels, round bottom flasks (amber), beaker, centrifuge tubes with stoppers, graduated test tubes (amber), condensers
- low volume air sampler or high volume air sampler
- filter paper folder : glass or stainless (teflon packing)
- electric furnace
- ultrasonic extractor (or sonicator)

III.2.4.5 Experimental

A. Sample preparation (note 1)

a) Water samples

Place 1 L of water sample into a separating funnel. Add 100 mL of hexane, and shake for five minutes, allow to settle, and collect the hexane layer. Add 50 mL of hexane to the water layer again, and repeat the procedure. Combine the hexane layers, dehydrate with anhydrous sodium sulfate, then reduce the volume of the solution to 5 mL using a rotary evaporator (keep the bath temperature below 50 °C). This extract is now ready for clean-up.

b) Sediment and biological samples

Place 20 g of sample (wet mud or crushed fish) into a round bottomed flask (amber). Add 100 mL of 1 M KOH-ethanol solution, and reflux the mixture for one hour. Allow to cool. After cooling, centrifuge at 4000 rpm, and collect the supernatant liquid. Transfer the supernatant liquid into a separating funnel. Add 400 mL of 4 % sodium sulfate solution. Extract the aqueous mixture first with 100 mL of hexane, then with 50 mL of hexane. Dehydrate the combined hexane layers with anhydrous sodium sulfate, then reduce the volume of the solution to 5 mL using a rotary evaporator (keep the bath temperature below 50 °C). This extract is now ready for clean-up.

c) Air samples

(1) Sampling

Attach a filter paper to the filter paper folder (high volume : 20 x 25 cm, low volume : 47 mmϕ) (note 3), connect a suction pump and a gas meter, pass air through the filter paper at the rate of 1300 L/min for high volume, 30 - 40 L/mm for low volume, for 12 - 24 hours to take air samples. Avoid having direct sunlight fall on the surface of the sampling area. Seal (wrap) the filter papers in aluminium foil after sampling.

(2) Extraction

Shred the filter paper. Put the shredded filter paper (note 4) into a centrifuge tube, add 1 mL of ethanol, then add 3 mL of benzene, and conduct ultrasonic extraction for 20 minutes. Immediately after the extraction, centrifuge at 3000 rpm for 15 minutes, and collect the supernatant for clean-up.

B. Sample clean-up

a) Water, sediment and living things

Load the 5 mL extract onto a silica gel column. First, elute the column with 100 mL of hexane (1st. Fr.). Discard the eluate. Next, elute the column with 50 mL of hexane- benzene (85 : 15 v/v), and elute target compounds (2nd Fr.) (note 5). Concentrate this 2nd fraction with a KD concentrator at atmospheric pressure to a volume of 2 mL. Reduce the volume further by blowing nitrogen gas across the surface of the solution until the sample solution reaches a final volume of 0.5 - 1 mL. This sample is used for measurement. If samples are measured by GC/MS, add 1 μg each of p-terphenyl-d_{14} and perylene-d_{12} as internal standards.

b) Air samples

Condition Sep-Pak cartridges by washing with 10 mL of hexane-benzene (9 : 1 v/v). Load 2 mL of extract onto the cartridge. Elute target compounds with 20 mL of hexane-benzene (9 : 1 v/v). Concentrate the eluates with a KD concentrator to about 2 mL. Reduce the volume further by blowing nitrogen gas across the surface of the solution until the sample solution reaches a final volume of 0.5 mL. This sample is used for measurement. If samples are measured by GC/MS, add 1 μg each of p-terphenyl-d_{14} and perylene-d_{12} as internal standards.

C. Preparation of blank samples

Blank samples should be prepared by subjecting purified water (1L for water, 20mL for

sediment and biological samples) to the same procedures used when examining analytical samples. For air samples, simply use the same kind of filter papers.

D. Preparation of standard solutions

Weigh 100 mg of each standard chemical accurately; add sufficient acetone to make an accurate 100 mL solution. This procedure gives a 1000 μg/mL standard stock solution.

- For HPLC analysis of B(j)F, dilute the standard stock solutions with methanol to make four different concentrations (1, 2, 3 and 4 μg/mL) which contain three standards of B(j)F, B(b)F and B(k)F.
- For HPLC analysis of the other target compounds, prepare four different methanolic solutions containing 0.05, 0.1, 0.15 and 0.2 μg/mL of B(e)P, and 0.1, 0.2, 0.3 and 0.4 μg/mL of the other seven compounds.
- For GC/MS analysis, prepare acetone solutions containing the eight compounds at five different concentrations, and add 1 μg of each p-terphenyl-d_{14} and perylene-d_{12} (add 0.1 mL of 10 μg/mL standard solutions).

E. Measurement

a) HPLC conditions

- column : Perkin-Elmer make PAH 2.6 mm x 250 mm
- guard column : Nucleosyl C18 4.6 mm x 33 mm
- column temperature : 30 °C
- mobile phase : methanol : water = 85 : 15 (note 6)
- flow rate : 0.8 mL/min
- detector : Ex 365 nm, Em 430 nm → B(a)P, B(b)F, B(k)F, B(ghi)P, [MC]
  Ex 300 nm, Em 393 nm → B(e)P, [B(k)F], B(ah)A, MC
  Ex 330 nm, Em 509 nm → B(j)F, [B(b)F], [B(k)F] (note 7)

b) GC/MS conditions (note 8)

(1) **GC : capillary column GC**

- column : ULTRA-2 (5 % phenylmethylsolicone chemical bonded type, 25 m x 0.32 mm i.d., film thickness 0.17 μm) or similar type of column
- column temperature : 50 °C (1 min) → 25 °C/min → 160 °C → 15 °C/min → 250 °C → 2 °C/min → 290 °C (5 min)

- injector temperature : 250 °C
- carrier gas : He (flow rate 40 cm/s)
- injection method : splitless (purge after 1 min)

(2) **MS : quadrupole type or double focusing type**

- transfer line temperature : 290 °C
- ionisation method : EI
- ionisation voltage : 70 eV
- ionisation current : 300 μA
- ion source temperature : 230 °C

(3) **monitor ion**

- target compounds : B(a)P, B(e)P, B(b)F, B(j)F, B(k)F = 252, B(ghi)P = 276, B(ah)A = 278, MC = 268
- internal standards : p-terphenyl-d_{14} = 244, perylene-d_{12} = 264

F. Calibration curves

For HPLC, inject 20 μL of the standard mixtures onto the column and record the detector response. Make calibration curves using the external standard method by plotting compound concentration against and peak areas (or peak heights). For GC/MS, inject 1 μL of standard mixtures onto the column. Make calibration curves using the internal standard method.

G. Determination and calculation

HPLC : inject 20 μL of sample and determine analyte concentrations by using the calibration curves (extrapolate from sample peak areas (or peak heights)) to sample concentration). GC/MS : inject 1 μL of sample and determine analyte concentrations by using the calibration curves of the internal standards and target compounds.

Calculate concentrations of each target compound from the following formulas.

a) Water , sediment, and living things

$$\text{concentration (mL or μg)} = \text{detected amount (ng)} \times \frac{\text{sample solution amount (mL)}}{\text{injection amount (μL)}} \times \frac{1}{\text{sample amount (mL or g)}}$$

b) Air

$$\text{concentrat ion (ng/m}^3\text{)} = \text{detected amount (ng)} \times \frac{1000 \times (273 + t)}{V \times (273 + 20)} \times \frac{760}{P} \times \frac{Vc}{I}$$

t : average temperature at the time of sampling (°C)

V : sampling air amount (L)

P : atmospheric pressure at the time of sampling (mmHg)

Vc : sample solution amount (mL)

I : injection amount (μL)

III.2.4.6 Recovery tests results

A. Results of recovery tests undertaken using the target compounds and water, sediment and biological samples are shown in **Table III-2-9**.

Table III-2-9 Recovery and relative standard deviation (RSD) of water, sediment and biological samples

Samples	recovery (%) (RSD (%))							
	B(a)P	B(e)P	B(b)F	B(j)F	B(k)F	B(ghi)P	B(ah)A	MC
river water	92 (4.8)	95 (5.7)	96 (3.8)	92 (4.5)	92 (4.0)	93 (3.4)	92 (4.3)	90 (3.6)
Seawater	94 (5.3)	95 (8.5)	93 (4.3)	94 (4.0)	85 (3.5)	95 (2.5)	94 (3.7)	94 (4.2)
Sediment	89 (3.7)	88 (4.0)	87 (5.0)	85 (8.3)	88 (7.5)	89 (8.0)	89 (6.8)	90 (6.7)
living things	90 (4.2)	87 (6.2)	89 (4.5)	90 (7.0)	92 (6.7)	90 (6.6)	88 (7.1)	87 (7.4)

water : n=4, sediment and living things : n=7

B. Results of recovery tests using the target compounds in air samples

Recovery measurements were undertaken by placing 0.5 μg of each target compounds onto a quartz filter papers(*1), and sucking indoor air through the filter paper (*2). Results are shown in **Table III-2-10**. The table shows that the recovery of these compounds decreases as amount of aeration increases. This is especially true for B(a)P and MC.

The recovery of B(a)P and MC was measured by aerating 3.0 m^3 of highly purified nitrogen gas at an aerating rate of 20 L/min. Also 3.0 m^3 of indoor air was aerated in the same way as a control. The results are shown in **Table III-2-11**. Though the recovery was 80 % when nitrogen gas was aerated, it was only around 50 % when indoor air was aerated.

(*1) 0.5 mL of standards (1 mg/L per one PAH) were added by using pipettes.

(*2) Particles in the air are removed by filter papers attached in front of filter papers for recovery.

Table III-2-10 Recovery and relative standard deviation of target compounds from filter papers depends on difference of aeration amount

aeration amount	B(a)P	B(e)P	BF	B(ghi)P	B(ah)A	MC
0.0 m^3	95.0 % (3.1 %)	93.5 % (12.0 %)	93.5 % (12.0 %)	91.0 % (12 %)	92.0 % (11.9 %)	78.0 % (11.7 %)
9.7 m^3	24.8	92.6	83.5	86.6	88.9	12.6
17.3 m^3	26.3	91.2	81.1	95.7	97.2	13.5
39.5 m^3	20.4	87.5	73.8	95.5	97.5	8.5

(Relative standard deviation (n=3))

Table III-2-11 Recovery of the target compounds from filter papers depends on aerating gases

aerating gases		B(a)P	MC
air (indoor)	recovery (%)	51.2	46.3
	RSD (%)	9.5	4.0
nitrogen	recovery (%)	85.5	80.2
	RSD (%)	2.5	3.1

aerating amount : 3.3 m^3, n=3

III.2.4.7 Notes

(1) B(a)P, MC etc. in aqueous solution or organic solvents are degraded by light. Therefore, samples and standard solutions should be shielded from light during storage and chromatography. For instance, MC in acetone at 100 μg/mL will be totally degraded in six months even if stored in amber test tubes.

(2) If fatty acids etc. from biological samples are deposited when concentrating the extract to 5 mL, in order to avoid clogging of columns centrifuge and collect the supernatants for clean-up.

(3) Carry filter papers wrapped in aluminium foils, and attach the filter paper folder at the sampling sites.

(4) Cut out the necessary size of filter papers for high volume air sampler for analysis and store the rest in the desiccator in a dark and cold place. Use the whole 47 mmϕ quartz filter paper for low volume air sample.

(5) It is necessary to check elution patterns beforehand. The eight target compounds can be completely eluted with 50 mL of 15 : 85 benzene-hexane mixture.

(6) If the separation of three isomers of benzofluoranthene is not sufficient, increase the amount of water in the mobile phase to improve resolution (for example, 20 : 80

H_2O-methanol). Methanol gives better separation than acetonitrile in the mobile phase.

(7) Divide the samples into three groups to minimise analysis time. Where it is possible to set the most appropriate wavelengths for each of the target compounds, use the following for measurement.

The most appropriate wavelength : B(a)P → Ex = 370 nm, Em = 407 nm, B(e)P → Ex = 335 nm, Em = 379 nm, B(b)F → Ex = 350 nm, Em = 395 nm, B(k)F → Ex = 370 nm, Em = 406 nm, B(ghi)P → Ex = 385 nm, Em = 419 nm, B(ah)A → Ex = 298 nm, Em = 395 nm, MC → Ex = 365 nm, Em = 420 nm

(8) The retention time order of the three isomers of benzofluoranthene is B(b)F, B(j)F, and B(k)F, but it is impossible to separate the baseline. If the presence of these three compounds in the sample is confirmed by GC/MS, then conduct quantitative analysis by HPLC.

III.2.5 Analytical method for aldehydes and ketones in air

III.2.5.1 Target compounds

acetaldehyde, formaldehyde, crotonaldehyde, acetone, 4-methyl-2-pentanone, 2-butanone

III.2.5.2 Outline of analytical methods

Collect air samples by sucking air into sample trap cartridges (trap agent : silica gel dipped with 2,4-dinitrophenylhydrazine (2,4-DNPH)). Target compounds react with the trapping agent and are held as hydrazones in the cartridges. Elute hydrazone derivatives from the cartridges by ethyl acetate, treat with cation exchange resin, and add internal standards after dehydration and concentration, then determine by GC-FTD (NPD).

III.2.5.3 Detection limits

Detection limits of this analytical method are shown in **Table III-2-12**.

Table III-2-12 Detection limits of this analytical method

compounds	detection limits (ng)
acetaldehyde	42[1)]
formaldehyde	4.4[1)]
crotonaldehyde	2[2)]
acetone	2[1)]
4-methyl-2-pentanone	2[2)]
2-butanone	2[2)]

1) values determined from the blank value change ($3\sqrt{2}\sigma$)

2) values determined from S/N ratio against interferences (S/N=3)

III.2.5.4 Reagents and apparatus

A. Reagents

- acetaldehyde : MERCK
- formaldehyde : special reagent grade
- crotonaldehyde : special reagent grade
- acetone : for spectroscopic analysis
- 4-methyl-2-pentane : special reagent grade
- 2-butanone : for ultraviolet absorption spectrum
- 2,4-DNPH form of target compounds : (note 1)
- diphenylamine (internal standard) : special reagent grade
- ethyl acetate : for HPLC (note 2)
- anhydrous sodium sulfate : special reagent grade. Heated in an electric furnace at 400 °C before use.
- hexane : for HPLC
- purified water : for trihalomethane analysis

B. Apparatus

- ozone scrubber : Waters chromatography Division / Millipore Corporation products. Form copper pipe (46 mm i.d., 1 m length) into coil (coil diameter is not specified), fill the coil with potassium iodide solution (dilute 1 g of potassium iodide into 0.7 mL purified water), and leave for 10 minutes. After 10 minutes remove the potassium iodide solution from the coil, and dry by nitrogen gas.
- sample trap cartridges : Waters Chromatography Division / Millipore Corporation products Sep Pak ® DNPH-Silica Cartridges (Short Body) (note 3). Silica gel which is filled up in the

cartridges is coated with 1.0 mg 2,4-DNPH. Discard the cartridges after use because the blank value of the cartridges increase depends on the storage duration (recommended storage length by the manufacturer is about one month at 20 - 25 °C, 6 months at 4 °C).

- cation exchange resin : TOSHO Corp. products TOYOPAK IC SP-M

III.2.5.5 Experimental

A. Sampling method

Connect the ozone scrubber, sample trap cartridge, suction pump, and gas meter, then suck the air sample through the apparatus and trap the target compounds. Sucking speed is at 0.1 - 1.0 L/min, and the sampled volume is 20 - 200 L. During sampling, shield cartridges by aluminium foil to avoid light. After sampling seal the both ends of the cartridges, place into glass containers with stoppers, and wrap with aluminium foil. Return the trap cartridges to the laboratory and conduct extraction procedures as soon as possible.

B. Sample preparation

After sampling, connect a sample trap cartridge to cation exchange resin, inject ethyl acetate by using a glass syringe, and elute the hydrazone derivatives from the cartridge (note 4). Place the eluate into an Erlenmeyer flask, add anhydrous sodium sulfate to dehydrate, filter into test tubes, and concentrate the hexane solution up to 1 mL by blowing nitrogen gas across the surface of the solution. Add 40 μL of internal standard for analysis.

C. Preparation of standard solutions

To prepare the stock solutions, weigh the masses of the hydrazone derivatives of each of the target compounds shown in **Table III-2-13**, and dissolve in ethyl acetate (50 mL). One mL of stock solutions contain 50 μg of each target compounds. Dilute and mix these stock solutions to make each target compound concentration 0.5 - 10 μg/mL. An internal standard stock solution is prepared by dissolving 20 mg of diphenylamine in 100 mL of ethyl acetate. Store both stock solutions and standards in light tight glass containers in a cold and dark place.

Table III-2-13 Amount of standards for stock solutions

compounds	amount (mg)
acetaldehyde-2,4-DNPH	12.7
formaldehyde-2,4-DNPH	17.5
crotonaldehyde-2,4-DNPH	8.9
acetone-2,4-DNPH	10.3
4-methyl-2-pentanone-2,4-DNPH	7.0
2-butanone-2,4-DNPH	8.8

D. Measurement

a) Analytical conditions of GC-FTD

- detection method : GC-FTD (NPD)
- column : fused silica capillary column ULTRA#1 (length 25 m x 0.32 mm i.d. x film thickness 0.17 μm) or similar column
- carrier gas : He, column head pressure 73 kPa
- column temperature : 50 °C (1 min) → 30 °C/min → 200 °C (15 min)
- injector temperature : 260 °C
- detector temperature : 280 °C
- injection method : splitless (purge after 1 min)

b) Analytical conditions of GC/MS (note 5)

- column : fused silica capillary column DB-1 (length 30 m x 0.25 mm i.d. x film thickness 0.25 μm) or similar column
- carrier gas : He, column head pressure 40 kPa
- column temperature : 50 °C (1 min) → 30 °C/min → (15 min)
- injector temperature : 260 °C
- separator temperature : 280 °C
- ionisation current : 300 μA

E. Calibration curves

Prepare standard solutions for calibration curves by adding 40 μL of internal standard to 1 mL of standard solution, inject 2 μL of the mixture into the GC, and make calibration curves by the internal standard.

F. Determination

Inject 2 μL of sample solutions into GC, determine peak area ratio of target compounds and internal standard, and decide detected amount (ng) from the calibration curves.

Calculate concentration of each target compound C (ng/m^3) from the following formula.

$$C\ (ng/m^3) = C' \times 1000 \times \frac{273 + t}{V + (273 + 20)} \times \frac{101.3}{P}$$

C' : detected amount (ng)

t : average temperature at the sampling time

V : collected air volume (m^3)

P : atmospheric pressure at he sampling time

III.2.5.6 Results of recovery tests

After connection of a trap cartridge and glass T-tube, 10 μL of hexane solution of each standard (concentration : 0.2 - 0.4 μg/μL, water solution for formaldehyde only) was injected. Nitrogen gas was passed through for more 15 minutes at the rate of 400 mL/min, then recovery was determined. **Table III-2-14** shows the results.

Table III-2-14 Recovery and relative standard deviation of recovery tests (n=5)

compounds	recovery (%)	RSD (%)
acetaldehyde	97	10
formaldehyde	95	22
crotonaldehyde	95	7.0
acetone	82	10
4-methyl-2-pentanone	81	4.0
2-butanone	84	4.1

III.2.5.7 Notes

(1) Commercial hydrazone derivative standards are sold by RADIAN (all 6 compounds) and Tokyo Kasei Corp. (5 compounds except 4-methyl-2-pentanone-2,4-DNPH).

(2) Check blank values of ethyl acetate and use ethyl acetate with minimum impurities (the blank values vary depending on manufacturers, grade or lot). Commercial reagent for pesticide residue analysis contains 0.08 - 1.2 μg/mL of acetaldehyde.

(3) Sep Pak ® DNPH-Silica Cartridge has a long body type which contains double the amount of adsorbent, and can be expected to provide more trap amount. However, it is recommended to use a short body type because of increase of impurities from solvent accompanied by increase of amount of solvent used.

(4) Reverse elution direction of cartridges from sample loading. Allow gravity and the weight of the syringe cylinder to inject ethyl acetate into cartridges. If solvent cannot be injected automatically, it is recommended to put a light weight on the cylinder. When silica gel becomes colourless after elution, extraction has completed (because 2,4-DNPH, which coats silica gel in the cartridge, moves as a yellow band).

(5) Conditions of GC/MS are for peak confirmation.

III.2.6 Analytical method for volatile compounds in water

III.2.6.1 Target compounds

Dichloromethane, dibromochloromethane, tetrachloromethane, trichloromethane (chloroform), tribromomethane (bromoform), bromodichloromethane, 1,2-dichloroethane, 1,1,1-trichloroethane, 1,1,2-trichloroethane, 1,1-dichloroethylene, cis-1,2-dichloroethylene, trans-1,2-dichloroethylene, tetrachloroethylene, trichloroethylene, 1,2-dichloropropane, 1,3-dichloro-1-propene, 1,4-dichlorobenzene, xylene, benzene, toluene

III.2.6.2 Outline of analytical method

Transport the target compounds from the aqueous phase into the gaseous phase by bubbling an inactive gas through the sample solutions. Trap the target compounds specialised trap cartridges, then heat the cartridges to desorb the target compounds, conduct cryo-focussing at the cooling and condensing apparatus, and introduce the target compounds into the GC/MS for measurement. Alternatively, after trapping the target compounds in the trap cartridges, heat the trap cartridges, then introduce the target compounds into the GC/MS directly (without cryo-focus).

III.2.6.3 Detection limits and determination limits

Table III-2-15 shows determination limits and repeat analysis accuracy of this analytical method.

Table III-2-15 determination limits and the accuracy of repeated analysis when using this analytical method

target compounds	range of determination (ng)	repeatability (%)
all compounds	0.5 – 250	10 - 20

III.2.6.4 Reagents

- target compounds : special grade or more, high purity reagents
- methanol : of a grade known not to contain target compounds
- blank water : water which doesn't contain target compounds (note 1)

III.2.6.5 Experimental

A. Sample preparation

Collect water samples directly into P&T vial bottles (note 2) at the sampling site, add 5 μL of p-bromofluorobenzene internal standard (20 mg/L) to the sample solutions.

B. Preparation of control

Use blank water, and conduct the analysis using the same procedures as used on the samples.

C. Preparation of standard solutions

a) Target compounds

Weigh 100 mg of each target compounds accurately, add methanol to make a precise 100 mL solution. This gives a 1000 mg/L standard stock solution. Dilute this stock solution with methanol, and prepare mix standard solution of concentrations 2, 20, and 50 mg/L.

b) Internal standards

Weigh 100 mg of p-bromofluorobenzene accurately; add methanol to make a precise 100 mL solution. This gives a 1000 mg/L standard stock solution. Dilute this stock solution with methanol, and prepare a standard solution of concentration 20 mg/L.

c) Preparation of standard solutions for measurement

Place about 30 mL of blank water into a P&T vial, add 10 μL of 2, 20, 50, and 100 mg/L

standards, then add 5 μL of p-bromofluorobenzene internal standard, and dilute with a known volume of blank water to produce standard solutions for measurement.

D. Measurement

a) Measurement conditions of P&T

- machine : ex. TEKMAR LSC-2000
- purge : 10 min at room temp
- dry purge : 4 min
- cryo cooldown : -150 °C
- desorb : 2 min at 220 °C
- inject : 3 min at 220 °C
- bake : 20 min at 260 °C

b) Measurement conditions of GC/MS

- machine: quadrupole MS, ex. JEOL-Automass 50 type
- column : ex. VOCOL (SUPELCO) 60 m x 0.32 mm i.d. x 3 μm

(1) **Conditions of GC**

- init. temp : 40 °C
- init. time : 5 min
- rate : 7.0 °C/min
- final temp : 230 °C
- final time : 5 min
- inject temp : 180 °C
- carrier gas flow rate: 1.0 mL/min (He)

(2) **Conditions of MS**

- emission current : 300 μA
- ionisation energy : -70 eV
- detection gain : -0.6 kV
- source temp : 210 °C
- interface temp : 230 °C

(3) **Monitor ions are shown in Table III-2-16.**

Table III-2-16 Monitor ions

target compounds	monitor ions
dichloromethane	84, 86, 49
dibromochloromethane	129, 127, 131
tetrachloromethane	117, 119, 121
trichloromethane (chloroform)	83, 85, 47
tribromomethane (bromoform)	173, 171, 175
bromodichloromethane	83, 85, 47
1,2-dichloroethane	62, 64
1,1,1-trichloroethane	97, 99, 61
1,1,2-trichloroethane	97, 83, 99
1,1-dichloroethylene	96, 61
cis-1,2-dichloroethylene	96, 61, 98
trans-1,2-dichloroethylene	96, 61, 98
tetrachloroethylene	166, 164, 129
trichloroethylene	130, 132, 95
1,2-dichloropropane	63, 76, 62
cis-1,3-dichloropropene	75, 110, 49
trans-1,3-dichloropropene	75, 110, 49
1,4-dichlorobenzene	146, 148, 111
o-xylene	106, 91, 105
m-xylene	106, 91, 105
p-xylene	106, 91, 105
benzene	78, 77, 52
toluene	92, 91
p-bromofluorobenzene	174, 176, 95

E. Calibration curves

Analyse standards by GC/MS by injecting 5 mL of standard solution into the P&T apparatus, and make calibration curves according to the relations between purge amount (μg) and peak area ratio of standards and internal standards.

F. Determination and calculation

Analyse samples by GC/MS by injecting 5 mL of standard solution into the P&T apparatus, and determine the detected amount from the calibration curves after determining the peak area ratio of target compounds and internal standards.

$$\text{calculated amount (μg/L)} = \frac{\text{detected amount (ng)}}{\text{purge amount (mL)}}$$

III.2.6.6 Notes

(1) To make blank water, boil purified water to about half its initial volume using a gas stove, and cool down by blowing helium gas across the surface of the water. When commercial water is used, check that target compounds are not detected.
(2) Check the capacity of the P&T vials before use because there are small differences around 43 mL in the case of Tekmar Co. products. Remove vials which have big error.

III.2.7 Analytical method of organochlorine pesticides in water, sediment and biological samples

III.2.7.1 Target compounds

Aldrin, α-HCH, β-HCH, γ-HCH (lindane), δ-HCH, p,p'-DDT, p,p'-DDE, p,p'-DDD, dieldrin, endosulfan I, endosulfan II, endosulfan sulphate, endrin, endrin aldehyde, heptachlor, heptachlor epoxide (isomer B), methoxychlor, trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor, toxaphene, hexachlorobenzene (HCB)

III.2.7.2 Outline of analytical method

- Extract water samples with hexane, dehydrate, concentrate, and then analyse by GC/MS-SIM or GC-ECD.
- Extract sediment samples with acetone, add sodium chloride solution, and then extract with hexane. After dehydrating and concentrating the hexane layer, clean-up by florisil column chromatography, and analyse by GC/MS-SIM or GC-ECD.
- Extract biological samples with a mixture of acetone and hexane, then wash with water. Remove lipids by acetonitrile-hexane partitioning, fractionate by florisil column chromatography, and analyse by GC/MS-SIM or GC-ECD.

III.2.7.3 Reagents and apparatus

A. Reagents

- organic solvents : pesticide analysis grade

- target compounds : commercial standard reagents
- internal standards (naphthalene-d_8, fluoranthene-d_{10}, perylene-d_{12}) : Cambridge Isotope Laboratory products
- anhydrous sodium sulfate, sodium chloride : pesticide analysis grade or reagent special grade, heated at 700 °C for 8 hours then cooled
- purified water and 5% sodium chloride water solution : wash twice with dichloromethane, and finally with hexane
- florisil : pesticide analysis grade (60 / 100 mesh), heated at 130 °C for 8 hours then cooled slowly in a desiccator
- 5 % hydrated silica gel : activate "Wako gel C-200", or equivalent, at 130 °C overnight, then cool slowly in a desiccator. Place 100 g of the silica gel into a Erlenmeyer flask fitted with a glass stopper, add 5 mL of purified water, replace the stopper, and leave for 4 - 5 hours. Shake occasionally until evenly mixed.
- charcoal : for column chromatography
- minute crystal powder cellulose : for column chromatography
- reduction copper : reduction copper for analysis of organic elements (60 - 80 mesh). Store under nitrogen gas, and wash with the solvent being used in the experiment immediately before use.
- other reagents : reagent special grade

B. Apparatus and instruments

- silica gel column : use a glass column (length, 30 cm; internal diameter, 1 cm) packed with 1 g of 5 % hydrated silica gel. Use hexane to add the silica gel as slurry. Lay 2 cm of anhydrous sodium sulfate onto the top of the silica gel. Wash with 20 mL of hexane which contains 2 % acetone before use.
- florisil column : use glass column (length, 30 cm; internal diameter, 1 cm) packed with 10 g of florisil. Use hexane to add the florisil as slurry. Lay 2 cm of anhydrous sodium sulfate onto the top of the florisil. Wash with 100 mL of hexane before use.
- activated charcoal column : use a glass column (length, 30 cm; internal diameter, 1 cm) packed with 5 g of cellulose which contains 10 % activated charcoal onto the 2 cm layer of anhydrous sodium chloride. Use hexane to add the charcoal as slurry. Lay 2 cm of anhydrous sodium sulfate onto the charcoal. Wash with 100 mL of hexane before use.
- Kuderna-Danish (KD) concentration apparatus
- rotary evaporator

- separating funnel
- homogeniser
- ultrasonic irradiation instrument (ultrasonic bath is also OK)
- centrifuge
- gas chromatograph / mass spectrometer (GC/MS) or GC-ECD : capillary column type GC, double focusing or quadrupole MS

III.2.7.4 Experimental

A. Sample preparation

a) Water samples

Dissolve 50 g of sodium chloride in 1 L of water sample, then extract with 50 mL of hexane by shaking for 10 minutes. Separate the hexane layer. Repeat the extraction. Combine the hexane layers, dehydrate with anhydrous sodium sulfate, concentrate by rotary evaporation up to about 5 mL, then concentrate the hexane solution to about 1 mL by blowing nitrogen gas across the surface of the solution. Use this solution for sample clean-up.

b) Sediment samples

Place 50 g of wet mud into a centrifuge test tube fitted with a glass stopper. Add 50 mL of acetone, and shake for 10 minutes. Conduct an ultrasonic extraction of the mixture by placing the sample in the ultrasonic bath for 10 minutes. Centrifuge the sample at 3000 rpm for 10 minutes, and collect the supernatant liquid. Repeat this procedure three times. Combine the supernatant liquids (the acetone extracts), and add them to 500 mL of 5 % sodium chloride solution in a 1 L separating funnel. Add 50 mL of hexane and shake for 5 minutes. Allow the organic and aqueous layers to separate, then collect the hexane layer. Repeat this procedure twice. Combine the hexane layers. Dehydrate the hexane solution by anhydrous sodium sulfate, concentrate by rotary evaporation up to about 5 mL, then concentrate the hexane solution to about 1 mL by blowing nitrogen gas across the surface of the solution. Use this solution for sample clean-up.

c) Biological samples

Add 50 mL of acetone and 100 mL of hexane to 50 g of homogeneous sample, and homogenise by polytron type homogeniser for 2 minutes. Centrifuge this at 3000 rpm for 10 minutes, then collect the supernatant liquid. Repeat this procedure twice. Combine the extracts in a separating funnel, add 150 mL of purified water, then shake gently (swirl or rotate the mixture). After settling, discard the aqueous phase. Repeat this water wash, then dehydrate the organic

phase by passing it through a column packed with 30 g of anhydrous sodium sulfate. Finally, concentrate by rotary evaporation to 10 mL. Place this concentrate into a separating funnel with 50 mL of acetonitrile saturated by hexane, shake for one minute, then collect the acetonitrile layer. Add 50 mL of acetonitrile saturated by hexane into the hexane layer and shake, then collect the acetonitrile layer. Combine the acetonitrile layers, add 5 mL of water, then shake gently. After settling, collect the hexane layer. Add the acetonitrile layer to 500 mL of 5 % sodium chloride solution in a separating funnel, add 50 mL of hexane, then shake for 5 minutes. Collect the hexane layer. Repeat this extraction twice, and combine the hexane layers. Dehydrate the hexane solution by anhydrous sodium sulfate, concentrate by rotary evaporation up to about 5 mL, then concentrate the hexane solution to about 1 mL by blowing nitrogen gas across the surface of the solution. Use this solution for sample clean-up.

B. Sample clean-up

a) Water samples

Load the concentrated sample onto a silica gel column, and elute target compounds with 30 mL of hexane which contains 3 % acetone (note 1 & 2). Concentrate the eluate samples by KD concentration to about 2 mL, then concentrate the hexane solution for GC-ECD analysis to exactly 1 mL by blowing nitrogen gas across the surface of the solution. For GC/MS analysis, add internal standard to each fraction then concentrate.

b) Sediment samples

Load the concentrated sample onto a Florisil column, and elute target compounds with 100 mL of hexane (Fraction 1) (note 3 & 4), 100 mL of hexane which contains 4 % diethylether (Fr. 2), and 150 mL of hexane which contains 15 % diethylether (Fr. 3) in that order. For GC-ECD analysis, concentrate each fraction to precisely 1 mL by KD concentration under reduced pressure and by blowing nitrogen gas across the surface of the solution (note 5). For GC/MS analysis, add internal standard to each fraction then concentrate (note 5).

c) Biological samples

Load the concentrated sample onto a Florisil column, and elute target compounds with 100 mL of hexane (Fraction 1) (note 4), 100 mL of hexane which contains 4 % diethylether (Fr. 2), and 150 mL of hexane which contains 15 % diethylether (Fr. 3) in that order. For GC-ECD analysis, concentrate each fraction to precisely 1 mL by KD concentration under reduced pressure and blowing nitrogen gas (note 5). For GC/MS analysis, add internal standard then concentrate (note 5).

C. Preparation of blank samples

Prepare blank samples as an analytical control by using the same procedures as described above for sample preparation and clean-up.

D. Preparation of standard solutions

Prepare 1000 mg/L standard stock solutions by dissolving standards of each target compound in hexane. Dilute and mix these with hexane to make standard mixtures of fixed concentrations of each analyte standard. Conduct the same procedure of preparation and storage for internal standard mixture solutions (naphthalene-d_8, fluoranthene-d_{10}, perylene-d_{12}) as used for target compounds.

E. Measurement

a) Measurement conditions of GC/MS

(1) GC

- column : fused silica capillary column (30 m x 0.25 mm i.d., 0.25 μm)
- liquid phase is methylsilicone or 5 % phenylmethylsilicone
- column temperature : 50 °C (1 min) - 10 °C/min - 280 °C (5 min)
- injector temperature : 250 °C
- injection method : splitless (purge after 1 min), injection amount 1 μL
- carrier gas : He, average linear velocity : 40 cm/sec
- transfer line temperature : 280 °C

(2) MS

- ionisation method : EI
- ionisation current : 300 μA
- ionisation voltage : 70 eV
- ion source temperature : 220 °C
- detection method : SIM

(3) measurement mass numbers (confirmatory ion)

- target compounds

Aldrin : 263 (265), α-HCH : 181 (219), β-HCH : 181 (219), γ-HCH : 181 (219), δ-HCH : 181 (219), p,p'-DDT : 165 (235), p,p'-DDE : 246 (318), p,p'-DDD : 165 (235), dieldrin : 263 (277), endosulfan I : 195 (241), endosulfan II : 195 (241), endosulfan sulphate : 385 (387), endrin : 81 (263), endrin

aldehyde : 67 (345), heptachlor 100 (272), heptachlor epoxide : 353 (355), methoxychlor : 227 (228), trans-chlordane : 373 (375), cis-chlordane : 373 (375), trans-nonachlor 407 (409), cis-nonachlor : 407 (409), toxaphene : 159 (231), hexachlorobenzene : 284 (286)

- internal standards

naphthalene-d_8 : 136, fluoranthene -d_{10}: 212, perylene-d_{12} : 264

b) Measurement conditions of GC-ECD

- column : fused silica wide bore capillary column (ex. : 30 m x 0.53 mm i.d., 0.5 μm) liquid phase is methylsilicone or 5 % phenylmethylsilicone
- column temperature : 50 °C (1min) - 8 °C/min - 280 °C (5 min)
- injector temperature : 250 °C
- injection method : direct injection, injection amount 3 μL
- carrier gas : He, aerate linear velocity : 40 cm/sec
- detector temperature : 280 °C

F. Calibration curves

When using the internal standard method, add prescribed amounts of internal standards into the standard mixture, take 1 μL from the mixture, and inject into the GC/MS, and make calibration curves based on the peak area (height) ratio of each target compounds and internal standards. When using the external standard method with wide bore columns, inject 3 μL of standard mixture into GC, and make calibration curves for each target compounds from the corresponding peak area (height). The concentration range covered by the calibration curves should be more than 5 orders of magnitude, including the minimum determinable concentration (which is anticipated to be close to detection limit of the analytical method).

G. Determination and calculation

When using the internal standard method for determination, inject 1 μL of sample solution into the GC/MS, and determine the detection amount from the calibration curve of peak area (height) ratio of each target compound and internal standards. When using external standard method for determination, inject 3 μL of samples to GC, and determine the detection amount from peak area (height) and the calibration curves of each target compounds. Then calculate concentrations of target compounds in the samples from detection amount, amount injected into the GC, analysed sample amount, and concentration rate etc.

III.2.7.5 Recovery tests

Conduct overall recovery tests using samples or similar samples per 10 samples or once a day, and calculate recovery rate. After adding acetone solution standards of target compounds at about ten times amount of detection limit and thoroughly mixing, leave for more than 60 minutes, then start the recovery tests.

III.2.7.6 Notes

(1) If there is no interference in analysis, column chromatography can be omitted.
(2) Check elution pattern of column chromatography and recovery rate of each compound. If the recovery rate is poor or interference appears, consider other column clean-up method (such as Florisil, silica gel, or alumina).
(3) Elemental sulfur elutes in the first fraction (Fr. 1) of Florisil column chromatography. If there is interference in GC analysis, add 2 g of reducing copper, shake vigorously for 1 minute, and then collect samples.
(4) PCBs elute in this fraction. If there is interference in GC analysis, conduct the following charcoal column chromatography : Load the concentrate of the fraction onto a charcoal column, elute with 100 mL of hexane and collect the eluate.
(5) Even if combining all fractions doesn't avoid GC interferences, it is OK to combine them. In this case, 150 mL of hexane which contains 15 % ethylether can be used for the elution from the beginning. Also add internal standards to the combined fractions.
(6) If target chemicals are detected by GC-ECD, confirm by using different polarity columns. It is desirable to confirm by mass spectrum in GC/MS analysis, but if it is not possible, measure more than two ions by SIM, then confirm from the peak intensity ratio.

III.2.8 Analytical method for tributyltin (TBT) and triphenyltin (TPT) in water, sediment and biological organisms

III.2.8.1 Outline of analytical method

(1) Water samples : Add tripentyl chloride as internal standard, and extract with hexane under acidic conditions. Separate and dehydrate the organic phase, concentrate, then propylise with bromopropyl magnesium. Clean-up the sample using a Florisil column, concentrate, and determine by GC-FPD.

(2) Solid samples : Add tripentyl chloride as internal standard, and extract with methanol-ethyl acetate under acidic conditions. Then extract the aqueous phase with ethyl acetate - hexane, and clean-up by anion and cation exchange resin. Then propylise in the same manner described above for water samples, and determine by GC-FPD.

III.2.8.2 Detection limits

Table III-2-17 shows detection limits of this analytical method.

Table III-2-17 Detection limits of this analytical method

target compounds	water sample		sediment and biological sample	
	sample amount	detection limits	sample amount	detection limits
TBT	1 L	2 ng/L	10 g	5 ng/g
TPT	1 L	6 ng/L	10 g	10 ng/g

III.2.8.3 Reagents and apparatus

A. Reagents

- organic solvents : pesticide analysis grade. If pesticide analysis grade is not available, use solvents which do not contain a GC interference peak with the retention times of both organotins, i.e. special reagent grade or higher.
- sulfuric acid, hydrochloric acid : special reagent grade or higher
- bromopropyl magnesium : 2 mol/L, tetrahydrofuran solution
- sodium chloride : special reagent grade
- anhydrate sodium sulfate : special reagent grade or PCB analysis grade
- tributyltin chloride (TBT)
- triphenyltin chloride (TPT)
- tripentyl tin chloride (TPeT) : as internal standard
- Florisil cartridge column : cartridge type (ex. Sep Pak Florisil)
- anion exchange resin : commercial cartridge type (note 1). Condition them with 10 mL of 0.2 M NaOH, 20 mL of purified water, and 20 mL of ethanol before use.
- cation exchange resin : commercial cartridge type (note 1). Condition them with 10 mL of 0.2 M HCl, 20 mL of purified water, and 20 mL of ethanol before use.

B. Apparatus

- rotary evaporator
- mechanical shaker
- mixer
- aspirator

III.2.8.4 Experimental

A. Sample preparation

a) Water samples

Measure 1 L of sample accurately. Place in a separating funnel. Add the prescribed amount of internal standard (e.g. 20 ng), 10 mL of hydrochloric acid, and 20 g of sodium chloride, and shake. Extract with 100 mL of hexane by shaking for 10 min. Separate the organic phase. Extract the aqueous phase with another 50 mL of hexane, then combine the hexane layers. Add anhydrous sodium sulfate to the hexane layer to dehydrate, swirl and filter. Concentrate the hexane solution under reduced pressure by rotary evaporation using a water bath at less than 40 °C until the final volume of the solution is about 5 mL. Place the concentrate in a test tube with a glass stopper and while heating gently, blow nitrogen gas over the surface of the solution to further concentrate the solution to about 1 mL. Use this sample solution for propylising.

b) Sediment and biological samples

Place 10 g of homogenised sample (biological sample are evenly ground with a mixer) into a centrifuge test tube, add the prescribed amount of internal standard (e.g. 20 ng) and mix thoroughly. Then add 70 mL of a mixture of methanol-ethyl acetate (1:1 v/v) containing 1 M hydrochloric acid, shake and extract for 30 minutes, then filter using an aspirator (note 2). Wash the test tube with a further 30 mL of the mixture of methanol-ethyl acetate (1:1 v/v) containing 1 M hydrochloric acid, and wash the residue by filtering using aspirator. Combine the filtrates and place them into a separating funnel. Add 100 mL of 10 % sodium chloride solution and 50 mL of ethyl acetate-hexane (3:2 v/v) and shake and extract for 5 minutes. Separate the organic phase. Repeat this procedure using another 30 mL of ethyl acetate-hexane (3:2 v/v). Combine the organic solvent layers in a separating funnel, add 150 mL of hexane, allow to settle for more than 20 minutes, and remove the aqueous phase (note 3). Add 100 mL of 10 % sodium chloride solution, and shake and wash the organic solvent phase. Repeat this procedure until pH of the aqueous phase becomes neutral (about four times) (note 4). After washing, dehydrate the organic solvent

phase by passing the mixture through a funnel packed with 20 g of anhydrous sodium sulfate. Collect the organic solvent in a round bottom flask. Concentrate the hexane solution under reduced pressure by rotary evaporation using a water bath at less than 40 °C until the final volume of the solution is about 1 mL. Then blow nitrogen gas over the surface of the solution until the solvent has evaporated completely. Dissolve the residue in about 10 mL of ethanol, pass through anion and cation ion exchange columns connected in series (anion exchange column first) at a flow rate of 1 mL/min to absorb organotin onto the cation column. Wash both columns with 20 mL of ethanol and remove the anion exchange column. Pass 15 mL of methanol solution containing 1 M hydrochloric acid through the cation column to elute the organotin. Collect the eluate in a separating funnel; add 30 mL of water and 5 mL of a mixture of hexane-cyclohexane (1:1 v/v), then shake and extract for 5 minutes. Separate and transfer the organic solvent layer into a round bottomed flask, and re-extract the aqueous phase with another 5 mL of hexane-cyclohexane (1:1 v/v). Combine the organic layers, concentrate the solution under reduced pressure by rotary evaporation using a water bath at less than 40 °C until the final volume of the solution is about 5 mL. Place the concentrate in a test tube with a glass stopper and while heating gently; blow nitrogen gas over the surface of the solution to further concentrate the solution to about 1 mL. Use this sample solution for propylising.

B. Sample clean-up (propylisation)

Add 1 mL of bromopropyl magnesium to the concentrated sample solution, mix by shaking gently, and allow to settle for 30 minutes at room temperature. Add 10 mL of 0.5 M sulfuric acid gradually and with external cooling. Transfer the mixture into a separating funnel, and add 10 mL of methanol and 10 mL of water. Extract this solution twice with 2.5 mL of hexane containing 5 % diethylether. Separate and combine the organic phase. Wash the organic extracts twice with 10 mL of water, and dehydrate the solution by passing the mixture through a funnel plugged by cotton or quartz wool and packed with anhydrous sodium sulfate. After washing a Florisil cartridge column with 10 mL of hexane, load the sample solution. Elute with 10 mL of hexane containing 5 % diethylether. Collect the eluate in a test tube fitted with a glass stopper (note 5). Concentrate the eluate by blowing nitrogen gas across the surface of the solution while heating gently to a final volume of 0.2 mL.

C. Preparation of blank samples

Prepare blank samples using purified water subjected to the same procedures as described

above for sample preparation and clean-up.

D. Preparation of standard solutions

Accurately and precisely weigh 20 mg of tributyltin chloride and 60 mg of triphenyltin chloride, and dissolve in 100 mL of hexane (standard stock mixture). Accurately dilute this stock solution 100 times with hexane to prepare standard mixtures (1.0 μg/mL tributyltin chloride and 6.0 μg/mL triphenyltin chloride). Prepare internal standard solutions of tripentyltin chloride (1.0 μg/mL) using the same two step procedure.

To a range of small volumes of the standard mixture (e.g. from 0.1 to 0.5 mL), add 0.5 mL of internal standard, and make up the solution to 1 mL with hexane. Then add 1 mL of bromopropyl magnesium solution to propylise, treat with 10 mL of 0.5 M sulfuric acid and 10 mL of purified water, then extract twice with 2 mL of hexane, and combine the extracts. Add hexane to the combined extract to make 5 mL. Use these standard solutions for preparation of calibration curves.

E. Measurement

Measurement conditions of GC

- column : fused silica capillary column (30 m x 0.53 mm i.d., 1.5 μm) liquid phase is methylsilicone
- column temperature : 80 °C (1 min) - 8 °C/min - 290 °C (10 min)
- injector temperature : 290 °C
- injection method : splitless or direct injection
- detector : flame photometric detector (FPD), with filter for tin, detector temperature : 300 °C

F. Calibration curves

Inject the standard solutions prepared for making the calibration curves into the GC. Measure the peak heights of the propylised forms of tributyl tin (TBT), tripentyl tin (TPeT), and triphenyl tin (TPT), then make calibration curves using the internal standard method and TPeT as internal standard.

G. Determination and calculation

Measure a constant amount (e.g. 3 μL) of samples by GC. Measure the peak heights of the propylised forms of TBT, TPeT, and TPT, and calculate the concentrations (as chlorinated compounds) of TBT and TPT in the samples using the following formula.

$$\text{TBT (TPT) (ng/L or ng/g)} = A \times \frac{\text{TPeT spiked amount (ng)}}{\text{sample amount (L or g)}}$$

A : absolute ratio of TBT (TPT) which is calculated from B values by calibration curves against TPeT

B : peak height (mm) of TBT (TPT) in samples / peak height (mm) of TPeT in samples

III.2.8.5 Recovery tests

Table III-2-18 and **Table III-2-19** show the results of recovery tests of water and biological samples.

Table III-2-18 Result of recovery tests (n=6)

Target compounds	river water			seawater		
	spike amount (ng)	recovery (%)	RSD (%)	spike amount (ng)	recovery (%)	RSD (%)
TBT	10	99.7	3.5	10	100.4	9.8
	100	98.4	2.2	100	96.3	4.2
	1000	99.0	4.3	1000	97.2	5.9
TPT	30	107.6	1.4	30	99.0	2.5
	300	98.7	1.9	300	97.5	7.4
	3000	107.3	4.1	3000	108.5	3.4

water sample amount : 1 L

Table III-2-19 Result of recovery tests (n=3)

Target compounds	biological samples		
	spike amount (μg)	recovery (%)	RSD (%)
TBT	0.5	90	1.9
TPT	1.0	77	3.9

sample amount : 10 g

Recovery of biological samples is measured by spiking propylised tripentyl tin immediately before GC analysis.

III.2.8.6 Notes

(1) Check recovery before hand because there are some differences depending on the kinds of ion exchange resin.
(2) Centrifuge if it is difficult to filter using an aspirator.
(3) Dehydrate with hexane increasing non-polarity because ethyl acetate content is large and it is difficult to dehydrate using anhydrous sodium sulfate.
(4) Wash with water thoroughly because the recovery of cation exchange resin becomes poor when acetic acid resulting from the hydrolysis of ethyl acetate remains.
(5) Florisil column clean-up can be omitted if there is no interference in GC analysis.

III.2.9 Analytical methods for the determination of organophosphorus pesticides in water, sediment, biological samples, and air

III.2.9.1 Target compounds

(1) water, sediment, biological organisms, and air : fenitrothion (MEP), diazinon, fenthion (MPP), edifenphos (EDDP), malathion, isoxathion, EPN
(2) water, sediment and biological organisms: kitazinon P, methidathion, salithion, phosalone, phosmet (PMP), α-chlorfenvinphos (α-CVP), β-chlorfenvinphos (β-CVP)
(3) air : dichlorvos (DDVP), propaphos, piperophos

III.2.9.2 Outline of analytical methods

(1) Extract water samples with dichloromethane, dehydrate, concentrate, add internal standard, then determine by capillary GC/MS-SIM or GC-FPD.
(2) Extract sediment samples with acetone, change solvent to dichloromethane, dehydrate and concentrate to dryness. Clean-up by hydrous silica gel column chromatography, concentrate, then determine by GC/MS-SIM or GC-FPD.
(3) Extract biological samples (e.g. fish) with acetone, change solvent to dichloromethane, dehydrate and concentrate to dryness. Then separate the non polar to relatively polar fractions and the polar fraction which contains lipids by silica gel column chromatography. Remove lipids from polar fraction by polyamide reverse phase chromatography. Extract the eluate from reverse phase chromatography with dichloromethane, and dehydrate. Combine

both fractions and concentrate, and determine by GC/MS-SIM or GC-FPD.

(4) Pass 1000 L of air samples through ODS packed trap cartridge using air sampler, then elute with a mixture of dichloromethane and n-hexane, concentrate and determine by GC/MS-SIM or GC/FPD.

III.2.9.3 Detection limits and determination limits

Table III-2-20 shows the GC/MS-SIM detection and determination limits for a range of organophosphorus pesticides in water, sediment and biological organisms. **Table III-2-21** shows the detection limits in air.

Table III-2-20 Detection limits and determination limits in water, sediment and biological organisms

compounds	water (μg/L)		sediment & biological organisms detection limits (μg/Kg)
	detection limit	determination limit	
salithion	0.013	0.042	2.4
diazinon	0.013	0.043	2.9
kitazinon P (IBP)	0.027	0.090	7.7
fenitrothion (MEP)	0.032	0.110	23.7
malathion	0.044	0.150	23.1
fenthion (MPP)	0.120	0.400	10.6
α-chlorfenvinphos (α-CVP)	0.023	0.078	11.7
β-chlorfenvinphos (β-CVP)	0.024	0.079	10.7
methidathion	0.072	0.240	37.5
isoxathion	0.110	0.380	N/D
edifenphos (EDDP)	0.034	0.110	35.2
phosmet (PMP)	0.048	0.160	27.3
EPN	0.120	0.390	18.6
phosalone	0.073	0.240	20.9

Table III-2-21 Detection limits in air

target compounds	detection limits (ng/m^3)
fenitrothion (MEP)	1.5
diazinon	1.0
fenthion (MPP)	1.0
dichlorvos (DDVP)	5.0
malathion	3.5
isoxathion	50
EPN	10
propaphos	1.5
piperophos	2.5

III.2.9.4 Reagents and apparatus

A. Reagents

- dichloromethane, acetone, hexane and methanol : pesticide analysis grade
- water : commercial mineral water (note 1)
- sodium chloride and anhydrous sodium sulfate : pesticide analysis grade
- azobenzene : reagent first grade
- pesticide standards : commercial pesticide standards
- internal: Cambridge Isotope Laboratory products
- eluent for polyamide column chromatography : methanol : water = 1 : 1
- silica gel : Wako gel C-200
- polyamide : polyamide C-200 (for column chromatography)
- preparation of 5 % and 40 % hydrated silica gel : activate "Wako gel C-200", or equivalent, at 130 °C overnight, then cool slowly in a desiccator. Place 100 g of the silica gel into an Erlenmeyer flask fitted with a glass stopper, add 5 and 40 mL of purified water, putting the stoppers in, and leave for 4 - 5 hours. Shake occasionally until evenly mixed.

B. Apparatus

- homogeniser : for biological sample extraction
- centrifuge : for separation of sediment and biological sample extracts
- Kuderna-Danish (KD) : sample concentration apparatus
- chromatography column : 1 cm i.d., 20 - 30 cm length
- ultrasonic extractor : for sediment sample extraction (ultrasonic bath is also OK)
- preparation of 5 % hydrated silica gel column for sediment : packed with 2 g of 5 % hydrated silica gel. Use hexane to add the silica gel as slurry. Lay 2 cm of anhydrous sodium sulfate onto the top of the silica gel.
- preparation of 40 % hydrated silica gel column for biological analysis : packed with 5 g of 40 % hydrated silica gel. Use hexane to add the silica gel as slurry. Lay 2 cm of anhydrous sodium sulfate onto the top of the silica gel.
- preparation of polyamide column for biological samples : packed with 1.0 g of polyamide in a chromatography column 1 cm i.d. using eluent (methanol : water = 1 : 1) to add the polyamide as a slurry (note 2). Liquid surface comes out about 2 cm on the column head (note 3).
- air sample trap cartridge : Sep Pak tC18 cartridge washed with 5 mL of dichloromethane :

n-hexane (1 : 1) and dried by passing ultrapure nitrogen gas through.

III.2.9.5 Experimental

A. Sample preparation

a) Water samples

Dissolve 50 g of sodium chloride in 1 L of aqueous sample in a separating funnel. Add 100 mL of dichloromethane, shake and extract. Separate the dichloromethane layer. Extract the aqueous phase again with another 50 mL of dichloromethane. Separate the organic phase and combine the dichloromethane layers. Dehydrate the dichloromethane layer with anhydrous sodium sulfate, and concentrate by KD concentrator (at atmospheric pressure) to a final volume of 3 to 5 mL.

b) Sediment samples

Place 10 g of sediment sample in a centrifuge test tube, add 30 mL of acetone, stir with a spatula and thoroughly disperse, and extract by ultrasonic for 10 minutes. After extraction, centrifuge at 3000 rpm for 10 minutes, and separate acetone layer. Add acetone to the residue, repeat extraction and separation, and combine the acetone layers. Add 200 mL of 5 % sodium chloride solution to the acetone layer, extract with 50 mL of dichloromethane twice, and combine the dichloromethane layers. Dehydrate the dichloromethane layer with anhydrous sodium sulfate, concentrate by KD concentrator to a final volume of 3 to 5 mL. Evaporate the solution to near dryness by passing a stream of nitrogen gas over the surface of the solution, then add 2 mL of hexane for clean-up. (note 4)

c) Biological samples

Place 10 g of biological sample in a homogeniser, add 30 mL of acetone, and homogenise for 5 minutes. Transfer the contents of the homogeniser to a centrifuge tube, centrifuge at 3000 rpm for 10 minutes, then separate the acetone layer. Repeat this extraction and separation with another 30 mL of hexane, then combine the organic phases. Add 200 mL of 5 % sodium chloride solution to the organic phase, extract with 50 mL of dichloromethane twice, and combine the dichloromethane layers. Dehydrate the dichloromethane layer with anhydrous sodium sulfate, concentrate by KD concentrator to a final volume of 3 to 5 mL. Evaporate the solution to near dryness by passing a stream of nitrogen gas over the surface of the solution, then add 2 mL of hexane which contains 500 ppm azobenzene for clean-up. (note 5)

d) Air samples

Connect a trap cartridge to a flow meter and an air sampler, pass air through the system at a

flow rate of 0.7 L/min for 24 hours, and sample 1000 L of air. After trapping the sample, connect syringe, pipette, etc. to the trap cartridge, put in 10 mL of a mixture of dichloromethane : hexane (1:1), and elute by passing through at the flow rate of about 1 mL/min without pressuring.

B. Sample clean-up

a) Water samples

Concentrate the prepared sample solution to 0.5 mL by passing a stream of nitrogen gas over the surface of the solution, and add prescribed amount of internal standards for analysis.

b) Sediment samples

Load the concentrate onto a 5 % hydrated silica gel column. Wash the column with 20 mL of hexane. Discard the eluate. Then elute the target compounds with 30 mL of hexane which contains 10 % acetone. Concentrate the eluate by KD concentration to a final volume of 3 to 5 mL. Evaporate the solution to 0.5 mL by passing a stream of nitrogen gas over the surface of the solution, and finally add a prescribed amount of internal standard for analysis.

c) Biological samples

Load the concentrate onto a 40 % hydrated silica gel column. Initially, elute the column with hexane, and note as the eluted volume the amount of hexane required to elute the centre of the yellow azobenzene band (azobenzene was added at the previous step to act as a marker for the column chromatography). Then continue eluting the column with hexane, and collect double the amount of hexane eluate recorded in the previous step (Bio. Fr-1) (note 6). Elute with 50 mL of hexane which contains 10 % acetone (Bio. Fr-2a). Concentrate fraction (Fr-2a) to a volume of 3 to 5 mL by KD concentrator, evaporate the solution to near 0.5 mL by passing a stream of nitrogen gas over the surface of the solution. Add 0.3 g of polyamide to the concentrate and stir with a spatula (note 7). Load this onto a polyamide chromatography column, develop with 120 mL of eluting solution, and collect the eluate in a container containing 100 mL of 5 % sodium chloride solution (note 8) (Bio. Fr-2b). Shake and extract the eluate / sodium chloride mixture with 30 mL of dichloromethane. Separate the dichloromethane phase. Repeat the extraction with another 30 mL of dichloromethane. Combine the dichloromethane phases, dehydrate with anhydrous sodium sulfate and filter. Combine the filtrate with the hexane fraction (Fr-1). Concentrate with a KD concentrator to a final volume of 3 to 5 mL. Evaporate the solution to 0.5 mL by passing a stream of nitrogen gas over the surface of the solution, and finally add a prescribed amount of internal standard for analysis.

d) Air samples

Add a prescribed amount of internal standard to the eluate, evaporate the solution to 0.5 mL by passing a stream of nitrogen gas over the surface of the solution. Use this solution for analysis.

C. Preparation of blank samples

Prepare blank samples using the same procedures as used for sample preparation and clean-up using the same amount of water as samples for the control. For blank air samples, operate the same procedures from the elution of a cartridge.

D. Preparation of standard solutions

Prepare 1000 mg/L standard stock solutions by dissolving each target compound in acetone. Mix prescribed amounts of standard stock solutions and dilute with acetone to make a 10 mg/L standards mixture. Dilute this mixture to prepare 1.0 mg/L solutions in acetone for standard mixture solution.

Internal standard solutions are each 1.0 mg/mL of phenathrene-d_{10}, fluoranthene-d_{10}, chrysene-d_{12} in acetone solution. Store these standards in a cool and dark place.

E. Measurement

a) Measurement conditions of GC/MS-SIM

- machine: ex. (MS) JEOL-DX303 DA-5000, (GC) HP 5790
- column : ex. fused silica capillary column Ultra-2-Crosslinked 5 % phenylmethyl silicone (25 m x 0.32 mm i.d., 0.52 μm) or similar column
- column temperature : 50 °C (1 min) - 5 °C/min - 280 °C (5 min)
- injector temperature : 250 °C
- interface temperature : 280 °C
- injection method : splitless (purge off ; 1.5 min)
- carrier gas : He (10 psi)
- ion source temperature : 250 °C
- ionisation energy : 70 eV
- ionisation current : 300 μA
- ionisation method : EI, Positive

- injection amount 1 μL
- motor ion : **Table III-2-22**

Table III-2-22 Monitor ions

Compounds	Monitored Ions
phenanthrene-d_{10} (internal standard)	188
fluoranthene-d_{10} (i. s.)	212
chrysene-d_{12} (i. s.)	240
fenitrothion (MEP)	277 (260)
diazinon	304 (179)
fenthion (MPP)	278 (169)
Edifenphos (EDDP)	310 (173)
malathion	173 (127)
isoxathion	313 (177)
EPN	185(169)
kitazinon P (IBP)	204 (288)
methidathion	145 (125)
salithion	216 (183)
phosalone	182 (367)
phosmet (PMP)	160 (317)
α-chlorfenvinphos (α -CVP)	323 (325)
β-chlorfenvinphos (β -CVP)	323 (325)
dichlorvos (DDVP)	220 (185)
propaphos	304 (220)
piperophos	320 (140)

(confirmatory ion)

b) Measurement conditions of GC-FPD (note 9)

- column : fused silica wide bore capillary column DB-5 Crosslinked 5 % phenylmethylsilicone (30 m x 0.53 mm i.d., 0.5 μm) or similar column
- column temperature : 50 °C (1min) - 5 °C/min - 280 °C (5 min)
- injector temperature : 250 °C
- injection method : direct injection
- carrier gas : He, flow rate : 10 mL/min
- injection amount : 3 μL
- detector : FPD (P mode)
- detector temperature : 280 °C

F. Calibration curves

For GC/MS analysis, take several volumes of the standard mixture, add prescribed amounts of internal standards, and add acetone or evaporate with nitrogen gas until the solution volume is 1 mL. Inject 1 μL into the GC/MS, and make calibration curves from the ratio of the peak area of the standards and the internal standard with the closest retention time.

For GC-FPD analysis, take several volumes of the standard mixture, add acetone to make exactly 1 mL, and inject 3 μL into the GC-FPD system. Construct calibration curves from peak areas.

G. Determination and calculation

Inject a known amount of sample (1 μL for GC/MS, 3 μL for GC-FPD).

- Internal standard method : determine the amount of target compound in the samples from the ratio of sample peak area and internal standard peak areas.
- External standard method : calculate the amount of target compound in the samples from calibration curves and gained peak areas.

Then calculate the concentrations in water, sediment and biological samples from formula 1. For air samples, use formula 2.

$$C\ (ng/mL\ \text{ or }\ ng/g) = \frac{C_S\ (ng)}{S\ (mL\ \text{ or }\ g)} \qquad \text{formula 1}$$

$$C_A\ (ng/m^3) = C_S\ (ng) \times \frac{1000}{V} \times \frac{273 + t}{273 + 20} \times \frac{1013.25}{P} \qquad \text{formula 2}$$

C : target compound concentration
C_S : target compound amount in the treated sample
C_A : target compound concentration in the air sample
S : Sample amount
V : trapped air amount (L)
t : average atmospheric temperature (°C)
P : average atmospheric pressure (hPa)

III.2.9.6 Recovery tests

A. Table III-2-23 shows the results of recovery tests performed using actual water, sediment, and fish samples spiked with target pesticides at low concentrations.

Table III-2-23 Recovery of water, sediment, and fish samples and relative standard deviation (RSD)

Compounds	river water			seawater		
	spike amount (μg)	recovery (%)	RSD (%)	spike amount (μg)	recovery (%)	RSD (%)
Salithion	0.12	101.5	3.3	0.12	121.3	5.7
Diazinon	0.18	122.0	2.3	0.18	115.8	5.4
Kitazinon P (IBP)	0.21	123.5	5.4	0.21	127.5	6.9
Fenitrothion (MEP)	0.3	116.0	1.2	0.3	115.3	5.4
Malathion	0.6	126.3	3.5	0.6	125.0	5.7
Fenthion (MPP)	0.3	112.5	1.1	0.3	108.3	5.3
α-chlorfenvinphos (α-CVP)	0.3	128.5	8.0	0.3	135.5	8.2
β-chlorfenvinphos (β-CVP)	0.3	125.0	7.2	0.3	128.3	5.8
Methidathion	0.9	121.5	7.5	0.9	125.8	6.0
Isoxathion	1.2	128.5	3.4	1.2	145.0	3.2
Edifenphos (EDDP)	0.9	125.3	2.1	0.9	122.0	4.7
Phosmet (PMP)	0.9	132.2	3.7	0.9	119.5	3.2
EPN	1.5	101.5	2.6	1.5	104.0	1.8
Phosalone	1.2	101.3	4.7	1.2	103.3	0.9
Compounds	sediment			fish		
	spike amount (μg)	recovery (%)	RSD (%)	spike amount (μg)	recovery (%)	RSD (%)
Salithion	1.0	102.3	17.7	1.0	39.7	5.1
Diazinon	1.0	97.5	6.2	1.0	91.8	4.7
Kitazinon P (IBP)	1.0	119.2	13.1	1.0	96.7	4.6
Fenitrothion (MEP)	1.0	113.0	25.8	1.0	99.5	6.6
Malathion	1.0	93.6	14.8	1.0	101.0	7.1
Fenthion (MPP)	1.0	109.2	21.9	1.0	97.5	4.7
α-chlorfenvinphos (α-CVP)	1.0	114.3	16.9	1.0	93.0	4.4
β-chlorfenvinphos (β-CVP)	1.0	116.7	15.7	1.0	90.9	4.2
Methidathion	1.0	133.2	20.4	1.0	94.4	5.0
Isoxathion (note 9)	-	-	-	1.0	66.6	35.3
Edifenphos (EDDP)	1.0	112.4	28.5	1.0	67.3	9.8
Phosmet (PMP)	1.0	113.2	6.4	1.0	11.3	23.9
EPN	1.0	125.6	11.7	1.0	91.1	10.8
Phosalone	1.0	166.2	15.5	1.0	85.9	8.7

water samples : 1000 mL, sediment and fish samples : 10 g

water sample : n=4, sediment and fish sample : n=7

B. Air samples.

Table III-2-24 shows the results of recovery tests in which 500 ng of each pesticide was dissolved in toluene, and then put onto a trap cartridge, then passed 1000 mL of air through for recovery tests.

Table III-2-24 Result of recovery tests of air samples

Compounds	recovery (%)	RSD (%)
fenitrothion (MEP)	104.8	3.8
Diazinon	111.5	4.2
fenthion (MPP)	68.0	4.9
dichlorvos (DDVP)	96.6	1.6
Malathion	109.9	4.2
isoxathion (note 9)	82.8	9.8
EPN	89.2	13.9
Propaphos	95.1	9.6
Piperophos	92.9	10.1

III.2.9.7 Notes

(1) Depending on the tap water source, distilled water made from tap water contains pesticides and other chemicals. Commercial mineral water is recommended. However, 2,6-di-tert-butyl-p-cresol (BHT) and its decomposition product, 2,6-di-tert-butylbenzoquinone, might be detected. These compounds seem to be derived from the container.

(2) Fine particles of polyamide come out and cause turbidity when preparing the column, but it will become clear after settling down.

(3) Leave a space of about 2 cm between the liquid surface and the column head so as to be able to fit in the polyamide sprinkled with sample.

(4) Depending on the sediment, it may be difficult to know when evaporation is complete -some of the contents don't evaporate when blowing nitrogen gas across the solution. In this case recovery may become poor. Try gentle heating during the blowing of nitrogen gas across the solution surface.

(5) When hexane elution has to be stopped right before an interference originating in the organism comes out, it is difficult to see the position of the interference. Therefore azobenzene is used as marker. Azobenzene is observed moving as a yellow band in the column. Graduated test tube (25 mL) is suitable for the collection vessel. Elute the azobenzene with about 7 mL of hexane.

(6) A small amount of target pesticide may be eluted in this fraction depending on solvent condition before column loading or the amount of fat. Besides start to measure amount of hexane at the point of sample loading.

(7) Elute with 120 mL of eluting solution. However, some interferences originating in the organism might be eluted with less than 120 mL depending on the sample conditions. If the sodium chloride solution in the collection vessel becomes cloudy, then stop development immediately.
(8) When GC-FPD seems to detect compounds, it is necessary to confirm this by using a column of different polarity.
(9) Consider insert cleanness or column conditions to analyse isoxathion because sensitivity of isoxathion is remarkably effected by dirtiness of injection port or column ageing.

III.2.10 Measurement method of coarse lipid

For many biological samples, it is desirable to express the concentration of target compound per unit volume or mass of lipid, as well as a simple sample concentration, because some types of target compounds, especially those compounds which have bioconcentration character (such as organic chlorine compounds), tend to be found primarily in lipid. This is done by measuring the total amount of coarse lipid contained in the sample.

Place 5 g of sample into a homogenizer cup. Add 20 mL of chloroform and 40 mL of methanol, and homogenise the sample for 2 minutes. Filter the resulting mixture. Add 80 mL of chloroform : methanol (1:1 v/v) to the residue, rehomogenise, and filter. Combine the filtrates (the organic layers) and transfer into a separating funnel. Add 60 mL of purified water, then mix by shaking gently. Collect the lower chloroform layer, dehydrate with anhyrous sodium sulphate, evaporate the solvent by rotary evaporation, then dry the sample in a desiccator using phosphorus pentoxide as desciccant. Finally, weigh the lipid sample. The results are to be expressed as lipid weight (%).

IV Evaluation of research results

Data contain errors which are derived from accidental, random, non-systematic and systematic characteristics of analytical methods. Environmental monitoring of chemical substances tends to investigate extremely low concentrations of analytes in various matrices with determination ranges at the ppb (parts per billion) level or less, so it is necessary to pay attention to the accuracy of the obtained values and the degree of precision by which they are obtained. Such details are described in the chapter about quality control. This chapter describes methods for the generalisation of the reality of pollution or symptoms, behaviour analysis, and risk assessment. The results which are dealt in this chapter are described on the assumption of data collected with guaranteed accuracy.

- Generalisation means processes which clarify the distribution of chemical substances, the character of any change, and find out the cause and regulation of determination of the concentration in the environment. This data treatment may make values which have no quality control problems unexplainable in reality, and this connects to processes of refining data.
- Behaviour analysis is the process used to understand data based on behavioural mechanisms of chemical substances in the environment. Here concepts such as material balance (incoming and outgoing) are also considered, mutual inspection between data and mathematical modelling is conducted, then matured mathematical modelling makes accurate prediction of future pollution possible.
- Risk assessment is the process used to clarify the reality of the kinds and sizes of risk of pollution caused by a chemical substance. Outlines of risk assessment to human health reported by NAS/NRC (National Academy of Sciences / National Research Council) have nearly reached international agreement, and they have been being used for indexing and the introduction of an index. The results gained from environmental monitoring are of particular use in policy decisions and implementation designed to decrease pollution, i.e. to develop risk management.

IV.1 Generalisation of research results

The results of environmental monitoring for chemical substances confirm if the target compounds exist in the environment, and at the same time check how much difference there is in

concentrations between survey points and regions, and the difference in concentration between multiple compounds. Environmental monitoring tries to understand in which media concentrations of target compounds will be large, e.g., water, air, living things, sediment, or soil. Furthermore, environmental monitoring aims to understand the essential features of concentration changes, and search for the causes by making temporal (time dependent) data plots, checking physiochemical characters such as water solubility and vapour pressure, and the relationship between data and environmental features such as wind direction, salt constituent, organic matters, etc. Such discussions are best dealt with by performing basic statistical procedures on the data, and visualising the data using graphs, bar charts and scatter plots, and also describing the reality of pollution by more convincing methods based on mathematical reasoning, comparison and correlation analysis etc.

Today, it has become easier to deal with complex data by the recent increase in personal computer memories and faster operating systems, the development and diffusion of superior spreadsheet, graphical, and statistics software. It is important to remember that data should be collated in an appropriate manner using software such as a spreadsheet. This not only facilitates data analysis but also accessing information on samples and field data such as sampling dates, water temperature, air temperature, climate, appearance, laboratory data such as the existence of interference, etc. and information on compounds such as molecular weight, boiling point, vapour pressure, water solubility etc..

IV.1.1 Feature of research results of environmental monitoring

Essentially, the results of environmental monitoring research is a collection of data. The data has a range. Data are usually shown as concentrations, but they are shown as “ND”, rather than given a numeric value, if they are less than detection limits of the analytical methods. Thus the data becomes a mixture of numeric concentration values and NDs. If the form of the distribution of each group of data cannot be assumed beforehand, it is possible to use normal distributions, logarithm normal distributions, or irregular distributions. However, data points termed outliers, “outside” and “far out” values, which are far apart from the general pattern of data distribution may have significant meanings. In addition, the number of data points is sometimes limited because of time and financial restrictions. Therefore, it is possible that data evaluation may change dramatically depending on the methods of data treatment, so it is often necessary to pay attention to data input to statistical methods and discard of huge amount of data.

IV.1.2 Basic statistical management

It is important to be able to grasp the shape of the distribution of data within a group in order to prevent the over- or under- estimation of the research results. Features of the data group have to be expressed by determining appropriate representative values and the degree of spread based on the shape.

IV.1.2.1 Making histogram

Divide the range between the biggest and smallest data points in a group into several even sections, and list how many data points are found in each section in a frequency table. Then make histogram in which the values defining the sections appear on the x-axis as the bases of the bars, and express the frequency of data appearance in each section as length of bars as **Figure IV-1-1**. In general sections are around 5 - 15. If the shape of histogram is symmetric and highest towards the centre, such as in the left figure of **Figure IV-1-1**, it is considered to have a normal distribution (or Gaussian distribution). If the shape of the histogram is symmetric when the values defining the sections of the bases of the bars in the histogram are logarithm transformed, the histogram has a log-normal distribution. Not only a single peak but multiple peaks may appear depending on the results of environmental modelling.

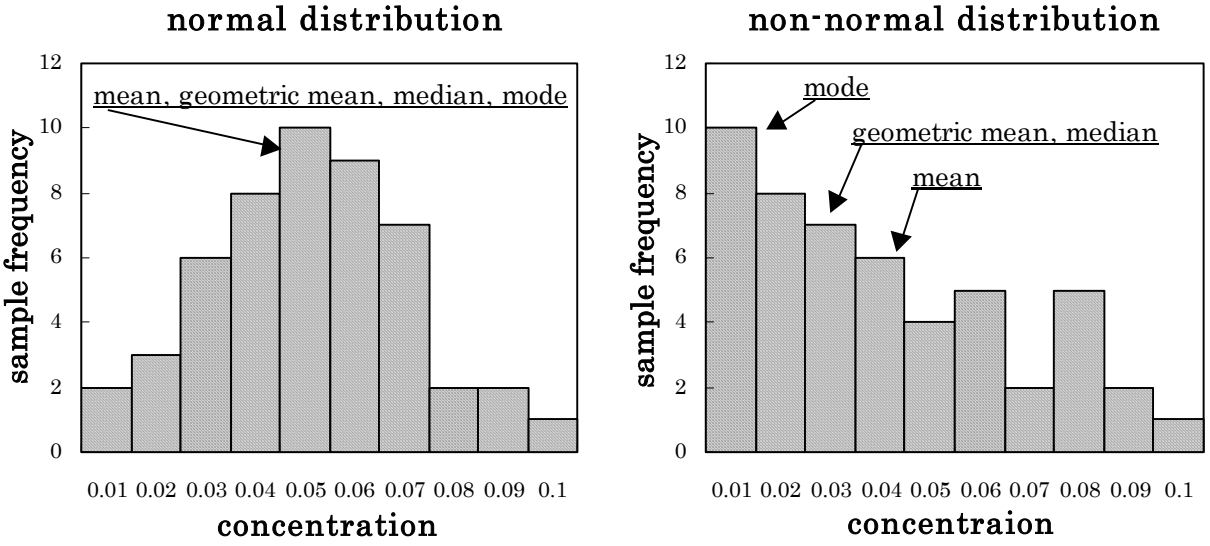


Figure IV-1-1 Example of histogram

IV.1.2.2 Representative values and the degree of spread

Chemical concentration data is often summarised as an average value in conjunction with the detection frequency. In addition, representative values and the degree of spread are used to compare the results obtained at different times, from different areas, compounds or samples. In this case, the kinds of representative values and the degree of spread are issues.

A. Detection frequency

Detection frequency is the percentage of the total number of analysed samples in which a target compound or compounds has been detected. This value is obtained by dividing the number of samples in which an analyte is detected by the number of analysed samples, shown as a percentage. This value becomes an index of the size and range of existence of the target compound in the environment, but doesn't show the range or level of concentration, because whether a chemical is detected or not depends on the determination limit of the analytical method.

B. Representative values

There are some basic, statistical quantities treated as representative values such as mean, geometric mean, median, mode, and trim mean, and these features are listed in **Table IV-1-1**.

Table IV-1-1 Kinds and features of representative values

representative value	definition	feature
Mean	values given by the sum of the data divided by the number of samples $X=(\Sigma x)/n$	values in which the difference to each data point has the smallest value.
Geometric mean	values given by the anti-logarithm of the mean of the logarithm of the data $X=10^{[(S\log)x/n]}$	used when data distribution is tailing towards the right
Median	values at the centre of the distribution of the data when the data is sorted in the size order. When sample numbers are even, take average of n/2 and (n/2)+1.	equal to mean when have symmetric distribution
Mode	the most frequent value or most frequent section	equal to mean when have symmetric distribution
Trim mean	remove values from the largest and smallest sides of the distribution at the same rate and take the average. removal rate is generally about 5 %.	underestimates or overestimates can be avoided

The values of the arithmetic mean, the geometric mean, the median and the mode (generally the 5 % trim mean is used. The 50 % trim mean equals to the median.) are almost the same if data are normally distributed. However, if the histogram is tailing towards the right, these values become "mode < median < mean", and tailing towards the left, "mode > median > mean". This illustrates the dependency of values on the shape of the histogram.

The mean is the value (of concentration, for instance) which shows the typical situation when symmetric normal distribution is assumed, and cannot be used where there is non-normal distribution. The geometric mean is applied to logarithm normal distribution. The median is different from these mean values, and it is a value which doesn't assume any distribution shape of data in the group, i.e. non-parametric value. Therefore it is convenient when distribution is irregular and there are a lot of "ND". The mode can be used as the median, but note that the mode is different depending on the width of section. The trim mean has characteristics of parametric and non-parametric methods, effects of outliers and far out values are small as well as the median because both ends of the distribution has been chopped off.

Occasionally, one must give "ND" a numeric value when calculating representative values, and it becomes a problem whether "ND" should be treated as zero or a certain number. Reality appears to be most appropriately reflected by considering the background level of target compounds in the environment, and to assign a numeric value to "ND" of around 1/2 - 1/10 of the background level when the background level is close to the analytical detection limits, and zero to 1/100 if it can be assumed there scarcely exists any target analyte in the environment at all.

C. The degree of spread

The degree of spread in a data set is the measure of the dispersion of the data, and is directly expressed as the maximum, the minimum, and the range between the maximum and minimum. The most common way to express the degree of spread is to use standard deviations. The standard deviation is the average difference in value of each data point from the mean value. The related basic statistical quantity, the variance, is square of the standard deviation. The coefficient of variation, or the relative standard deviation, is the standard deviation divided by the mean, and is a kind of relative error, and used in order to compare groups whose unit or size are different.

In non-parametric methods, the degree of dispersion is described by the interquartile range which replace the standard deviation, and are usually used with the median. When 'n' pieces of data are sorted in the size order, the data of n/4 and 3n/4 are quartile. The bigger number is called

the upper quartile, and smaller number is the lower quartile. The difference between the upper and lower quartile is the interquartile range, and the half is called the interquartile deviation.

D. Data representation as the box-and-whisker plot

One way to represent data, including outliers (out side and far out values), is the box-whisker plot. This is also called the box graph, and looks like **Figure IV-1-2**. The hinge spread is equivalent to the interquartile deviation. Determine the median and the quartile in the same way as the degree of spread in parametric methods. The upper quartile is called the upper hinge or the 75 % value, and the lower quartile is called the lower hinge or the 25 % value. The gap between the upper and the lower hinges is expressed as a box, divided the box by a line which is median. Stretch whiskers from the box to the data closest to the inner fence which is [the upper hinge]-1.5x[the hinge spread] and [the lower hinge]-1.5x[the hinge spread]. Also, the outer fence is [the upper hinge]-3x[the hinge spread] and [the lower hinge]-3x[the hinge spread]. Data which is outside of the inner fence and inside of the outer fence are termed the 'out side values.' Data which is outside of the outer fence are the 'far out values.'

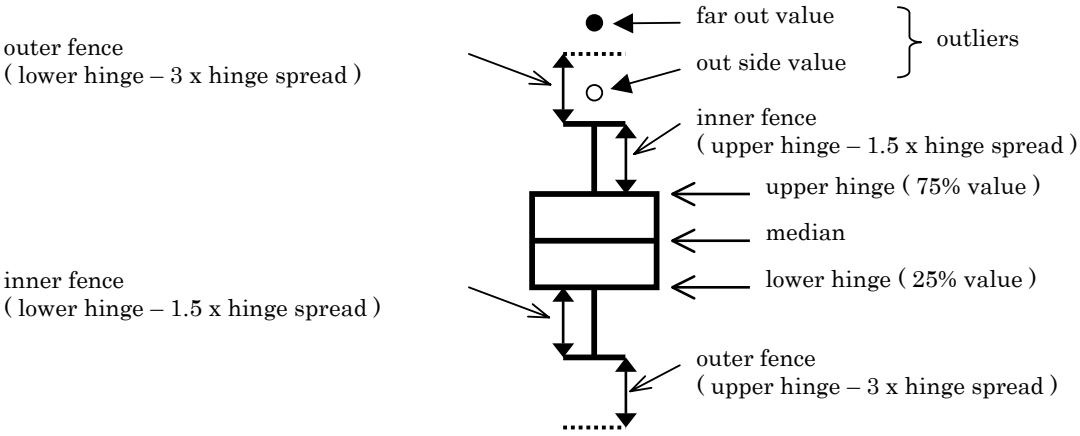


Figure IV-1-2 Box-whisker plot

IV.1.3 Visualisation of the research results

It is very useful to express research results as figures and graphs. This is done to gain an intuitive understanding of the contents. There may be lots of things to show, but in the case of environmental monitoring, observations on the distribution, chronology and correlation of results are indispensable.

IV.1.3.1 Distribution chart

Draw a distribution chart in order to understand how concentrations vary depending on the sampling point or region. Indicate data as circles or bars at the sampling point in the map. Connect sampling points (samples) which have the same concentration by curved lines (equal concentration lines).

IV.1.3.2 Chronological graph

There is the chronological graph in order to understand chronological, seasonal or yearly tendency of concentration level to change. The basic chronological graph is shown as the distribution chart which is plotted time, month or year on the x axis and concentration on the y axis.

IV.1.3.3 Correlation graph

This is mainly used when searching for the causes of concentration change. Plot data on the xy coordinates to determine the relationship of two parameters which may causes the change in concentration, e.g. two substance concentrations, or a single substance concentration and another parameter. These parameters are considerable: production and use quantity, distance from the expected pollution source, physiochemical characters such as water solubility, octanol partition coefficient, Henry's constant etc., environmental information such as temperature, water temperature, wind direction, salt amount, amount of organic matters etc., and furthermore, as useful parameters, biological measurement data such as body weight, body length, age, fat containing amount etc. Draw a line or curve through the data points, an obtain an appropriate regression formula. In general, the best method to gain the regressive of a straight line is to minimise the sum of the squares of the residuals. There amy be important cases where the regressive is a function other than that of a straight line. In this case, the function has to be explained to be applied.

IV.1.4 Significance test

There are many instances of comparison and data sorting, such as concentration levels and regional comparison of distribution situation etc., during evaluation of the research results of environmental monitoring. If there are no duplicate significant errors in representative values

and the degree of spread, it is easy to discover the size relationships. However, there is a problem when the representative values are different and the degree of spread has doubled. In such cases, statistical methods are needed to make data comparison convincing. Therefore, go back to the distribution of data within a group again. The shape of histogram can be categorised like **Figure IV-1-3**.

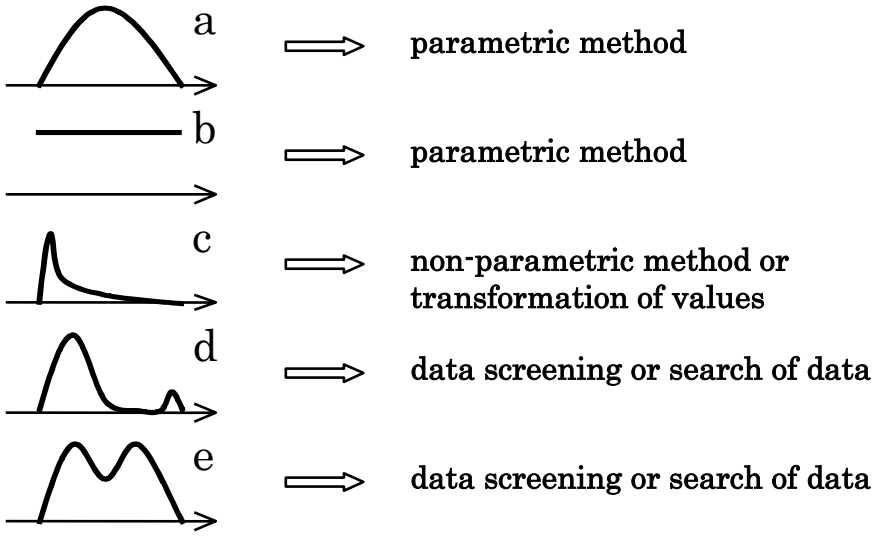


Figure IV-1-3 Classification of histogram and the application for statistical analysis

IV.1.4.1 Test of comparison of data group

The parametric method may be applied if the data has a normal distribution or there is no difference in the distribution like a) and b) in **Figure IV-1-3**. Although c) is for non-parametric methods, their normality has to be checked after changing data to its logarithm values. The geometric mean comes into existence when symmetry is gained. In cases where outliers or multiple peaks exist, like d) and e), it is necessary to re-check data , including repeating the research, and investigate the causes such as conditions of sampling, pollution sources etc.

Therefore, the flow chart of comparison test of two data groups is shown in **Figure IV-1-4**. **Figure IV-1-5** shows comparison test of more than three data groups.

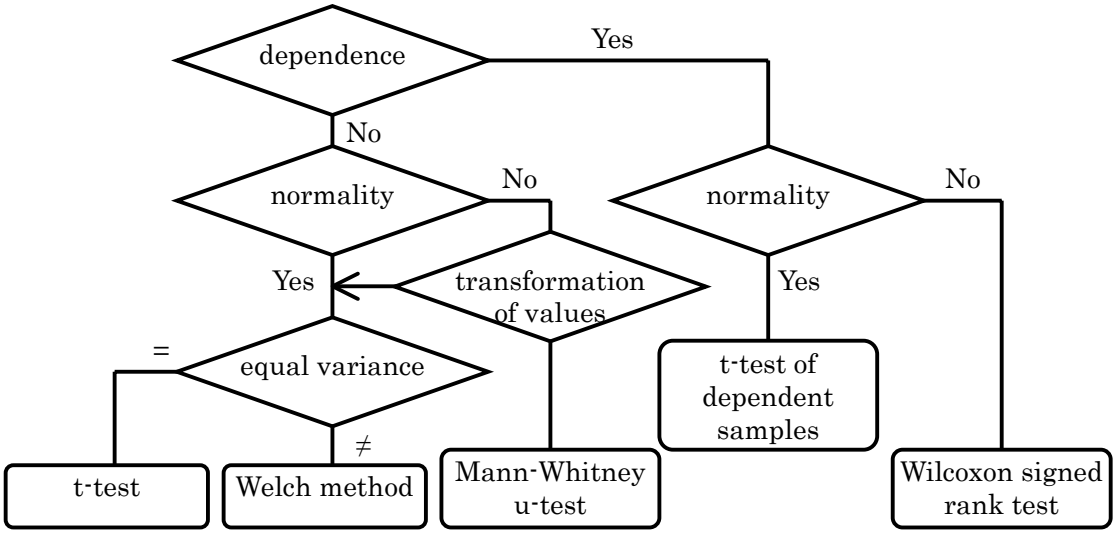


Figure IV-1-4 Flow chart for comparing two groups of data

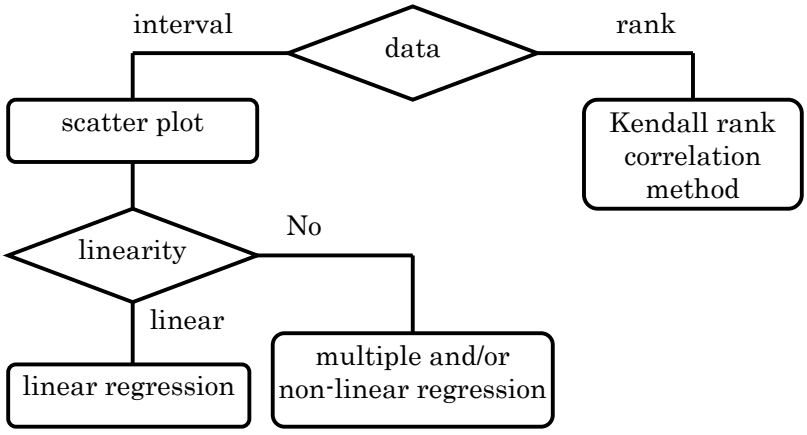


Figure IV-1-5 Flow chart for comparing multiple groups of data

IV.1.4.2 Correlation and regression

Regression formula which is obtained by correlation analysis and correlation coefficients also must be tested for significance (**Figure IV-1-6**).

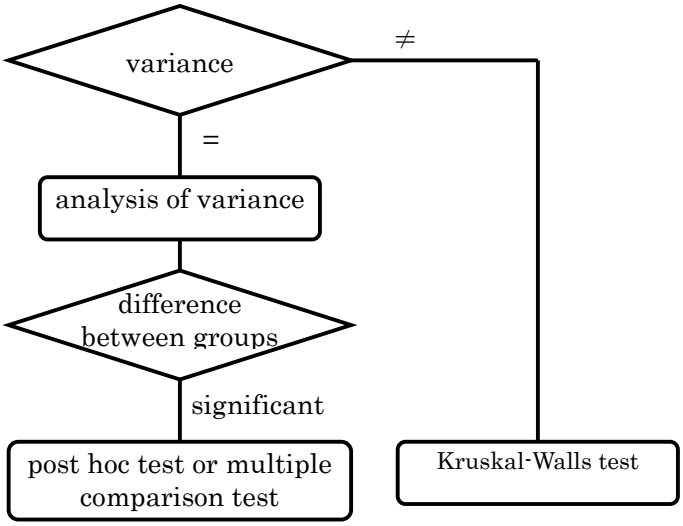


Figure IV-1-6 Flow chart of correlation analysis

IV.2 Behaviour analysis

Chemical substances which enter the environment move and diffuse between the atmosphere, water, soil and sediment, degrading gradually, and finally distribute themselves in the lowest energy levels (see **Figure IV-2-1)**. This movement is determined by reciprocal actions between such factors as human action, materials and the environment, and has a certain regularity. For example, chemicals in water are partitioned e.g. adsorbed onto suspended particles, accumulated by fish or other aquatic organisms, depending on their solubility in water and octanol-water partition coefficients, and the ratios of the concentrations in the various matrices become relatively stable values. Conversely, for water and biological samples taken at the same time, if the results show extremely different concentration ratios, it may be that the data from the water or the biological samples are wrong. In addition, the concentration of a chemical in a matrix can be estimated even if data has not been obtained for such samples or media, if the regularity is obtained. In this section, we show factors related to effects which are estimated to be happening

in the environment. Mentioned under the heading of generalised correlation analysis, it makes it possible to evaluate research results more accurately by examining data which have been obtained from environmental monitoring by referring each effect to the behaviour of chemicals.

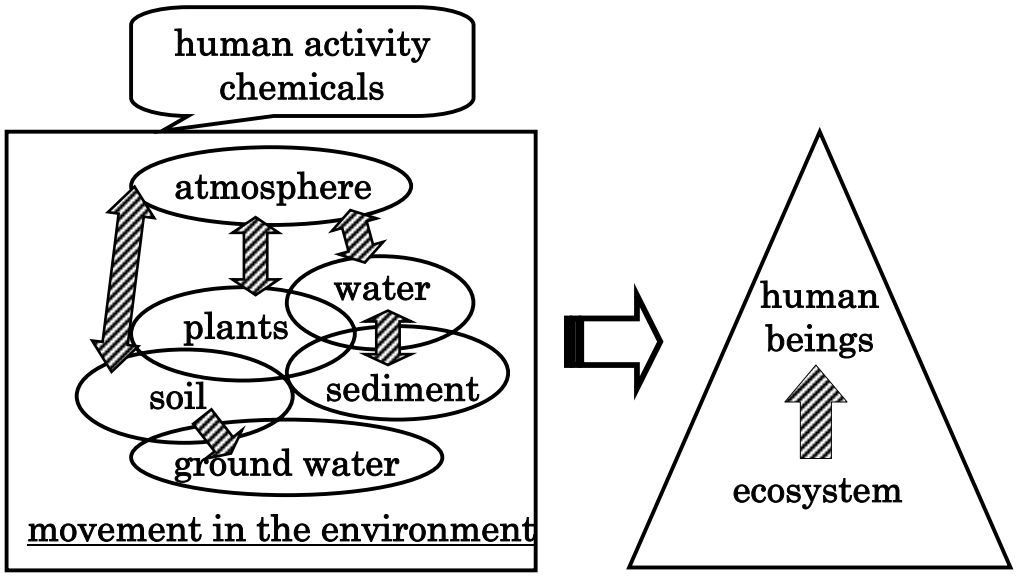


Figure IV-2-1 Concept of chemical movement in the environment and risk flow

IV.2.1 Information needed for behaviour analysis

Though it is very difficult to collect all pertinent information, parameters about chemical behaviour are listed. In the section covering risk assessment, useful resources are listed e.g. data bases and documents etc.

IV.2.1.1 Human action or social factors

Basically this information becomes useful for estimating, directly or indirectly, the flow or amount input into the environment.

- production and use amount
- use and purpose
- situation of use and abolition : distinction of open + closed system, raw materials + products, burning + reclamation etc.

IV.2.1.2 Substance factors

These are physical, chemical, and biological characteristics peculiar to chemical substances, can be used to estimate a compound's movement, partition, and degradation character in the environment.

- ultraviolet-visible absorption spectrum : determination of wavelengths which may be affect photodegradation
- melting point / melting point range : a factor which is affected by the condition of the chemical
- boiling point / boiling point range : a factor which is affected by the condition of the chemical
- vapour pressure (curve) : probability of phase change to air, estimation of evaporation rate, and atmospheric concentration
- water solubility : amount of elution to aqueous phase, concentration in water, estimation of adhesion and adsorption
- soil adsorption / desorption : exudation and evaporation from soil, adsorption to soil, the amount partition to the soil, and estimation of speed of travel in soil
- octanol-water partition coefficient : indicates the potential of a chemical to accumulate into living things
- Henry's law constant : estimation of evaporation from water surface to the atmosphere
- photodegradation character : possibility of decomposition by light in the atmosphere, soil and water surface
- hydrolysis character : possibility of decomposition in water
- microorganism decomposition (biodegradation) character : possibility of decomposition by microorganisms in soil or water
- bioconcentration coefficient : degree of bioaccumulation

IV.2.1.3 Environmental factors

These are the characteristics of the environmental conditions and the samples at sampling, and become information showing sample characteristics

A. Water quality

- climatic condition
- water temperature
- water flow (flow speed)
- water depth

- sampling point (surface - bottom)
- general water quality (pH, salt, suspension particles, organic pollution)
- location of discharge source, discharge condition etc.

B. Atmospheric air quality

- climatic condition (temperature, wind direction, wind speed, humidity, rainfall, weather forecast etc.)
- floating suspended particles
- location of discharge source, discharge condition etc.

C. Soil and sediment

- climatic condition
- temperature
- general character (pH, water content, organic matters content, oxidation-reduction potential etc.)
- soil character
- particle size distribution
- existence of living things
- use history, etc.

D. Living things

- biospecies
- biomeasurement data (body length, body weight, etc.)
- eating habits and feeding activities
- habitat, character of activity
- nutrition stages
- life and death and health conditions
- life expectancy, age
- sex
- growth stages
- reproduction conditions
- lipid amount and composition of tissues and organs
- location of discharge source, discharge condition etc.

IV.2.2 Mechanisms of chemical movement in the environment

Figure IV-2-2 shows the movement of chemicals in and between each environmental phase. Bearing these processes in mind, behavioural analysis is used to understand how each of the factors mentioned in the previous section are related, and to find out the regularity between single or multiple factors and data. In addition, it makes it possible to estimate the environmental fate and concentration of chemicals beforehand by using models of generalised behaviours.

It must be understood that chemicals discharged into the environment after production, use, and abolition follow processes of movement, transport and decomposition peculiar to the substance , which are influenced by various environmental conditions, and shift to a distribution equilibrium in which the more stable forms of the material form. As the result, the concentration and distribution of chemicals are determined, but the media making up the environment, such as the atmosphere and water, is always changing, and many processes related to chemical movement and disappearance in the environment exist. Furthermore, the environmental behaviour of chemicals changes in time and space because these process depend on various environmental conditions.

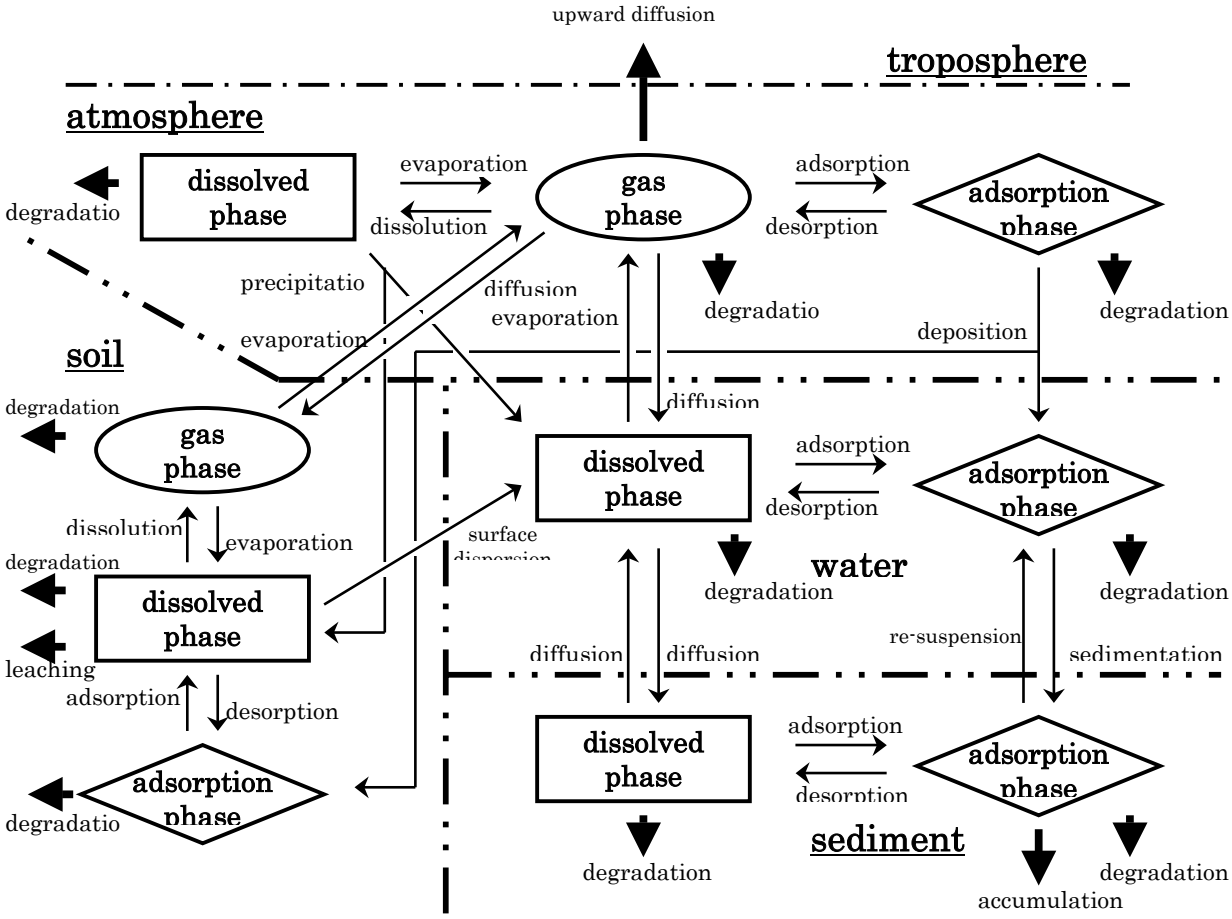


Figure IV-2-2 Movement and fate of chemicals in the environment

IV.2.3 Process of transport, movement and decomposition in the environment

There are many process which affect chemical behaviour in the environment. The degree of effect on chemical behaviour of each process in the environment depends on the character of the chemicals, the medium into which the bulk of the material is discharged, and environmental conditions. Therefore, it is important to understand environmental chemical behavioural processes and the conditions which control the speed of chemical movement.

IV.2.3.1 Transport processes

Transport processes relates to chemical movement in the environmental media, mainly advection and dispersion in the atmosphere, water, and soil. Function of these advection and dispersion in these media are basically the same.

A. Advection

Advection plays an important role in the environment. Chemicals in the gas phase and dissolved phase in the media are transported by advection. The speed of transport of chemicals by advection is given by the following formula.

$$N = G \times C$$

where G is the flow rate of environmental media (m^3/h), and C is the chemical concentration in the media (mol/m^3). Measurement of flow rate can be used for the calculation of the advection speed of a river or lake. Atmospheric flow rate calculated from wind speed etc. is used for air. Advection of substances in the gas phase and dissolved phase in soil is controlled by the vertical flow of air and water. Generally, the flow of air and water in soil is very small, but contributes to chemical transport which is controlled by the air and water compartments in the soil.

B. Turbulent flow dispersion

Turbulent flow dispersion plays an important role in the dilution of chemicals in the environment, and it is more important in the environment than molecular diffusion. Dispersion speed depends on the relative size of a turbulent flow swirl and plume which contains many chemicals. If the plume passes a place of advection, the speed also contributes (on what?). Dispersion speed is affected by wind speed and temperature etc., and is of the order 10 - 1,000 m^2/s. Turbulent flow dispersion is defined as the amount of chemical advection (J) in proportion to

concentration slope (C).

$$J = D \times \Delta C$$

or

$$dc/dt = d(Di \times dc / di) / di$$

where i is defined as the x, y, or z planes, and Di is the dispersion coefficient of these directions (m^2/s). The formula above can be integrated exactly, but in this case a simplified formula is used. Although there is a basic formula for turbulent flow dispersion in the atmosphere, it is impossible to predict chemical dispersion in the atmosphere accurately. In practice, distribution of chemical concentration by turbulent flow dispersion in the environment is shown by a Gaussian normal distribution using a dispersion width (standard deviation) which is a function of distance from the source and climatic conditions.

In surface water, the speed of movement of substances (Ri) by turbulent flow dispersion is shown by the following formula.

$$Ri = \Delta C \times Di \times Ai / i$$

where, i is defined as the x, y, or z planes, Di is the dispersion coefficient of these directions (m^2/s), A is exchange sectional area, l is the distance, and ΔC is the concentration slope of the x, y, and z directions. Dispersion happens in underground water, but is regarded to be a very small contribution to chemical transport.

C. Leaching

Leaching is a chemical transfer process which accompanies water flow in a vertical direction in the soil. The amount and direction of movement of underground water in soil is determined by the soil osmosis coefficient. The smaller the diameter of soil particles are, the smaller the coefficient is, and the smaller the permeation character. Therefore, when a clay layer which contains small diameter particles stays in the ground, ponding on the surface can be observed. Generally, the leaching coefficient (Kpe) is given by following formula:

$$Kpe = E \times ADS^2 \times g \times \rho / \mu$$

where E is a coefficient related to the air capacity of the soil, ADS is the average particle diameter of the soil, g is the acceleration due to gravity, and ρ and μ are the density and viscosity

of water, respectively.

IV.2.3.2 Transfer processes

Transfer processes between environmental media are important processes which control the distribution of a chemical in the environment. For example, if a system consists of surface water and sediment, evaporation, adsorption, sedimentation of suspended particles, re-suspension, sedimentation rate etc. are the main transfer processes. The main problem with transfer processes is how obtain representative velocity data for these processes. Estimation of such data for still water and slow flowing rivers is relatively straightforward, but river mouth and coastal zone processes are often difficult.

A. Evaporation from water

The thermodynamic equilibrium of chemicals between the surface of the water and the atmosphere is well understood, but the dynamics of volatilisation from inside the bulk of the water to the atmosphere has not yet been clarified. When chemicals in the air and water are in an equilibrium state, the chemical concentration in water (C_{wat}) can be related to the concentration of the same chemical in the air (C_{air}) by Henry's law.

$$C_{air} = H \times C_{wat}$$

where, H is a dimensionless value known as Henry's law constant. This Henry's law constant can be measured, and estimated fairly accurately from a chemical's vapour pressure and solubility in water. The speed of evaporation of a substance from inside a body of water depends on not only the nature of a substance, but also on the turbulent flow of water and the atmosphere. Various theories have been suggested, but the double thin layer theory of Whitman is most often used. This theory assumes two thin layers border both sides of the atmosphere and the water, and chemicals pass through the border layers by molecular diffusion. Therefore, the speed of evaporation depends on the nature of molecular diffusion in water and the atmosphere, and thickness of the border layers. In addition, the speed of evaporation can be determined by comparison with oxygen which is well investigated about evaporation from inside of water. The relations between flow speed or wind speed and evaporation speed.

B. Evaporation from soil

Evaporation from soil to the atmosphere is more complicated than evaporation from inside water, and it is dependent on chemical partitioning between air, water and particles in soil, and the

speed of diffusion from soil air to the atmosphere. When the water content of soil covers all of the soil particle surface, partitioning between air and particles in soil can be expressed as the two processes of partitioning between air and water, and between water and particles.

$$C_{air} = H \times C_{wat}$$

and

$$C_{sol} = Kd \times C_{wat}$$

where, C_{sol} is the concentration in particles, and Kd is the adsorption constant between particles and water. From these formulae, the equilibrium partition coefficient between air and particles in the soil can be expressed as the ratio of H and Kd..

$$C_{air} = H/Kd \times C_{sol}$$

Because there is no convenient formula to describe the speed chemical partitioning in soil, assuming partial equilibrium in the soil phase evaporation from soil is expressed by considering that chemicals partitioned into the soil air move into the atmosphere diffusively. The speed of evaporation from soil is largely affected by wind speed, soil temperature and gap ratio?.

C. Adsorption to soil and sediment

The adsorption of nonpolar chemicals to soil or sediment particles is regarded as partitioning to the organic materials in the particles, and the partition equilibrium is expressed using the adsorption constant, Kd, defined above. An adsorption constant (Koc) which takes into account the soil organic carbon ratio (%oc, %) can be used as a standard value, and can be applied to soil whose organic carbon content is known. Koc and Kd can be related by the following formula.

$$Koc = Kd \times 100/\%oc$$

Koc of chemicals such as pesticides have been reported, but those of most of chemicals are unknown. Therefore, for most models, Koc has to be estimated from the octanol-water partition coefficient and water solubility of the compounds of interest. Koc should not be applied to soil with low organic carbon content or high clay content because in such cases adsorption to inorganic matter becomes important.

The details of the dynamics of movement between water and particles have not yet been elucidated. One can consider adsorption dynamics being proportional to the concentration difference between solution and adsorbent, but this does not coincide with experimental data which generally shows rapid initial intake, followed by equilibrium.

The partitioning behaviour of ionic compounds, including materials such as metals and phenols, is not as clear as that of nonpolar compounds. Salt concentrations and pH are considered to affect the partitioning of ionic compounds between water and particles, but a complete partition theory doesn't yet exist. When the environmental fate of ionic compounds is explained by modelling, it is necessary to pay attention to these limits.

D. Bioconcentration

Bioconcentration is evaluated by comparing how high chemical concentrations are in living things with the concentration of chemical in the environmental media which surrounds the living things. Bioconcentration occurs by chemical uptake directly from media and/or via the food chain. Bioconcentration rate (BCF) is defined by the following formula:

$$BCF = C_{biota} / C_{wat}$$

where, C_{biota} is the concentration of chemical in living things. For nonionic compounds, BCF can be estimated from the compounds' octanol-water partition coefficients. However, the BCF of compounds which are easily metabolised and/or biologically decomposed, and high molecular compounds (>700 g/mol) can be overestimated. In general, the degree of bioconcentration is controlled by a compound's physiochemical character and its stability at the low nutrient stage, and an organisms' food habit, life span, degradation and metabolism ability, and physiological conditions.

E. Transfer accompanied with precipitation

Gaseous chemicals in the atmosphere are dissolved in rain drops and fall to earth with precipitation. The air-liquid equilibrium or transfer efficiency of such processes for nonpolar compounds can be estimated from Henry's law. Raoult's law can be applied for water soluble compounds. The half life of removal from the atmosphere by precipitation ($R_{1/2}$) can be determined from a chemical's concentration in rain drops obtained from chemical's vapour pressure (p, torr) and water solubility (x) expressed as mole fractions, and concentration ratio in the atmosphere (a).

$$R_{1/2} = 2310 / a$$

Generally, the smaller Henry's law constant is, the more compounds are dissolved in rain drops, and the more easily the compound moves from the atmosphere to the ground.

F. Transfer associated with deposition of particles in the atmosphere

Gaseous chemicals in the atmosphere are adsorbed to particles and move to earth when particles are deposited on the ground. Generally, deposition of particles larger than 1 μm is basically the result of gravity, and the speed of fall can be determined from Stokes law. The average life span of particles in the troposphere is said to be about 7 days. When the adsorption rate of atmospheric chemicals to atmospheric particles is ϕ, the life span of the chemicals in the atmosphere becomes 7/ϕ.Unfortunately, the adsorption rate ϕ is almost never measured, but only approximately estimated by the following formula:

$$\phi = \chi \times \theta / (\chi \times \theta + p)$$

where, θ is surface area of particles (cm^2) per 1 cm^3 of atmosphere, and χ is a coefficient (cm x torr) which is determined from the following equation:

$$\chi = 5.05\ (D/M)^{2/3}$$

where, D and M are density (g/cm^3) and molecular weight (g/mol), respectively. The smaller the vapour pressure of a compound is, the more the compound is adsorbed to particles and moves from the atmosphere to the ground. Generally, any compound with a vapour pressure less than 10^{-7} torr is easily adsorbed to particles and is deposited.

G. Dry deposition

Gaseous chemicals in the atmosphere also move from the atmosphere to other media by being adsorbed by soil, surface water, or terrestrial plants after coming into contact with them. Such a process is called dry deposition. Dry deposition occurs because adsorption onto, for instance, the earth's surface causes a layer of low concentration of compounds, and thereafter a concentration gradient is formed which causes further molecules of the compound to flow vertically downwards. The speed of this dry deposition is dependent on characteristics such as the ground surface, climatic conditions, and the nature of the chemicals, and varies quite considerably

H. Transfer associated with settling of suspended particles

Settling of suspended particles in water is an important process involving chemical transfer towards sediments for chemicals which are readily adsorbed by the particles. The settling speed of suspended particles can be estimated by using Stoke's law, in the same manner as deposition of

particles from the atmosphere. Partitioning of chemicals in water towards suspended particles is dependent on the chemical adsorption constant K_{oc} and the organic carbon content ratio of the particles.

I. Resuspension

Resuspension of sediment particles is an important transfer process that re-introduces chemicals into the water column. Particles attached to the sediment a the sediment-boundary layer resuspend if they obtain sufficient energy from water flow or biological disturbance. However, it is extremely difficult to estimate the speed of resuspension. If the accumulation speed is known, resuspension can be estimated form the difference between the rates of sedimentation and accumulation.

J. Burial

The surface of sediment is mixed by biological disturbances. The thickness of this region of sediment mixing is dependent on the degree of biological activity. Burial is the difference between the speed of sedimentation of suspended particles and the speed of resuspension. If sedimentation speed is bigger than resuspension, sediment particles are accumulated and the sediment layer becomes deep enough to avoid biological disturbance. Chemicals adsorbed by particles in this sediment layer do not readily re-enter the water column. Burial speed is slow, usually of the order of mm per year. The method which is the most commonly used to estimate burial speed is an ageing measurement.

K. Transfer diffusion between water and sediment

Diffusive transfers of chemicals can not only be by such processes as dry deposition from the atmosphere to soil, but also in the reverse direction i.e. evaporation from soil to the atmosphere, or between sediment pore water and the water column. The speed of diffusive transfer from sediment to water is dependent on nature of the surface of the sediment, the rate of water flow, the character of chemicals, etc. On the other hand, diffusion from sediment to water is dependent on partitioning between sediment pore water and chemicals within particles, and the speed of diffusion from pore water to the water column.

L. Surface runoff

Chemicals partitioned in soil pore water and adsorbed on particles are moved by rain into aquatic environments such as rivers. Surface runoff is a transfer process associated with flow from soil to the water system. Generally, in soil chemicals are partitioned between the soil air, soil water, and soil particles. The water content of the soil increases after rain fall. Water is held in soil up to the soil's saturation point, but if this limit is exceeded, water starts to flow on the surface of soil towards lower geographical points. at saturation, chemicals adsorbed to soil particles dissolve into water, and flow into rivers etc. with the water. It is not easy to estimate the fraction of water in soil, but usually such parameters are obtained by equating rain fall with an amount of water that equals the sum of the amount of water evaporating from the soil, surface runoff, water intake by plants, and ground water leaching.

M. Erosion

Erosion is a transfer process that generally has water assisting the movement of soil particles into rivers etc. Such particulate transfer is more common for small diameter clay particles than silts and sands. The degree of chemical transfer by erosion is dependent on intensity of rain fall and diameter of soil particles etc.

IV.2.3.3 Degradation Processes

Degradation is an important means by which chemical resides in the environment are controlled.

A. Biodegradation

Biodegradation of chemicals is a metabolic process, and is important in many situations. In general, if biodegradation of chemicals is confirmed in the laboratory, it will also occur in the wider environment. However, many of the dynamics of such processes are unknown, and biodegradation rate constants determined in the laboratory are not always applicable in the field. Many fate and exposure model users arbitrarily assign a value to the rate constant or use the following formula:

$$rb = \mu_{max} \times C \times X/(kb + C)$$

where, rb is the rate constant, μ_{max} is the highest growth speed, X is microorganism

concentration, kb is the concentration of substrate which causes a growth of half the highest growth speed, and C is chemical concentration. Under normal environmental conditions, the concentration C is very small, and the above formula can be simplified to:

$$rb = kb' \times C$$

In aqueous, soil, and sediment phases, biodegradation is the most important process. The rate of biodegradation is related to the structure of chemicals, the density of microorganisms, their carbon content, temperature, and also humidity in soil. It is difficult to estimate biodegradation rates accurately, but it is generally assumed in models to be a pseudo linear reaction. When, under special conditions such as in the deep soil or sediment, or surface water, oxygen content is extremely high anaerobic biodegradation is important, and growing microorganisms utilise nitric acid and sulphuric acid as an oxygen source. However, the dynamics of this process is little understood.

B. Hydrolysis

Hydrolysis is defined as the fission of organic molecules by reaction with water, for instance in the following formula:

$$RX + H_2O = ROH + HX$$

Typical functional groups which can be hydrolysed are halogenated alkyl groups, amides, amines, carbamates, epoxides, and esters. Hydrolysis can be separated into three different processes i.e. neutral, acidic, and alkaline reactions. Humic substances, metal ions, Brønsted acids, bases, etc. catalyse hydrolysis. In soils and sediments, hydrolysis is affected by adsorption onto particle surfaces. Because hydrolysis is pH dependent, data on the dynamics of hydrolysis at different pH is required for making models. In general, the pH of river water is in the range 4.5 - 8.5, sea water, 7.5 - 8.5, and soil, 4.5 - 6.5, but sometimes the pH can be as low as 3, or as high as 10 depending on the places.

C. Photodegradation

Photodegradation is categorised into direct and indirect reactions. Direct photodegradation occurs when chemicals absorb sunlight (>295 nm) directly, and react in the resulting excited states. Direct photodegradation reactions occur in many places in the environment, and with a range of the speeds. Indirect photodegradation occurs when chemicals react with unstable compounds, such as hydroxyl radicals, which have themselves been produced by the energy of sunlight.

In the troposphere, indirect photodegradation by reaction with hydroxyl radicals is the most important reaction. The reactivity of many chemicals with hydroxyl radical has been measured. For compounds for which there is no data, reactivity can be estimated by Atkinson's method. For some groups of compounds, reaction with ozone and nitric acid radicals is also important.

In water, direct photodegradation is important. Direct photodegradation is a result of compounds absorbing sunlight, and becomes an important process for compounds which have high quantum yields in water. Reaction rates depend on pH, chemical concentrations, dissolved oxygen, and especially transparency to light. Transparency to light is a function of water depth, concentration of suspended particles, and the colour of the water. Direct photodegradation rates in water are can be calculated using the following equation：

$$rp = kp \times C$$

where, rp is the photodegradation rate, kp is a pseudo linear reaction kinetic constant, and C is the chemical concentration in water.

For compounds which react with oxygen, such as compounds containing sulphur, indirect photodegradation is the more important process. High reactivity compounds in water are produced by photochemical reactions with dissolved humin. Oxygen, hydrated electrons, peroxides, and hydroxyl radicals are the main reactants. Hydroxyl radicals are also produced by light e.g. by irradiation of nitrate or nitrite solutions in the presence of metal catalysts.

In soil photodegradation is not regarded as important degradation process because it happens only at the soil surface.

IV.2.4 Behaviour evaluation model

As previously mentioned, the behaviour of chemical substances in the environment is associated with their transport, transfer and degradation mechanisms. In addition, the velocity or equilibrium status of each process is enormously affected by not only the chemical characteristics of substances, but also by the environment or climatic conditions. Models evaluate such behaviour by inserting environmental discharge data into chemical material (or mass) balance equations. By solving these equations, it is possible to obtain chemical concentration and distribution data, or residue information which are changeable in time and space. Data from single or multiple models of environmental discharges, behaviour / path ways in the environment, and exposure routes are used in early stage exposure analysis for the evaluation of potential chemical distribution in the environment. Such analysis is useful when estimating exposure concentrations under different environmental conditions, for the preparation of monitoring plans, or analysis of research results.

Furthermore, the importance of models is increasing, with several models of chemical behaviour and exposure in the environment having been developed and used with toxicity data to evaluate risk to humans and the harmfulness to other organisms in the environment. The problem is how accurately data related to each behaviour process, such as equilibrium constants or velocity constants, can be obtained. Even if the model is theoretically superior, results obtained using inaccurate data are meaningless.

Several of the models of environmental behaviour which have been already developed differ in their treatment (description) of the environment, and the processes which are considered. For instance, the methods of estimating equilibrium constants and velocity constants are sometimes different, because they rely heavily on the purposes of the models or developers' ideas. These differences ultimately appear as differences in the data output for each model.

Currently, there are many proposed models for the evaluation of chemical behaviour in the environment. When these models are classified by their target environmental media, they can be divided into two categories - single and multi-media models. The former aims to evaluate chemical behaviour in a single medium such as atmosphere, water or soil, while the latter evaluate chemical behaviour in more complex, multiple media (or compartment) environments. When applying these models, one must consider not only the media, but also sources of the target chemicals and the size of the environment. From this point of view, multimedia models often look at a wide range of behaviours on national or global scales. Therefore, sources of chemical discharges into the environment are naturally generalised, and the results themselves become more generalised. On the other hand, single media models aim for a more detailed evaluation of chemical behaviour in a localised environment, such as places close to discharge sources. Environmental modelling of chemicals basically seeks to discover whether targeted compounds exist in the environment at the national scale. Therefore, this chapter will focus on multimedia models, and, adopting the classification, criteria and composition of multimedia models, the MNSEM (Multi-phase Non-steady state Equilibrium Model) which was developed in Japan is introduced.

IV.2.4.1 Basic composition of models

Models generally consist of three programs - data input, calculation and output.

Input data comprises chemical characteristics, environmental conditions, and environmental discharge data. The chemical characteristics required for many models are physiochemical characters such as molecular weight, water solubility, vapour pressure, octanol-water partition coefficient, etc., and the degradation kinetic constants of biodegradation, hydrolysis, oxidation and photodegradation, etc. Physiochemical characteristics can be obtained from experiments, the

literature, or structure and activity relationships, but sometimes reliable data is difficult to find.

The environmental conditions required are such things as the capacity of media, height / depth, temperature, organic carbon content in particles, wind speed, rain fall amount etc. Chemical behaviour in the environment becomes complex if these environmental conditions change significantly in time and/or space. Many models use data which are averaged across time and space, but it is necessary to understand that these averaging may render uncertain important environmental chemical behavioural characteristics. In general, models which target local areas requires more environmental data.

The environmental discharge data required includes the rate of chemical discharge speed into the wider environment or each local medium. However, data covering chemical discharge into different environmental media are often inaccurate, estimated values. In many cases, discharge is not constant but discrete and often discontinued, so practical, averaged values are used.

Models calculate the distribution, chemical persistence, and chemical concentration in the environment by solving material balance formulae using input data. Therefore, models are advantage able to evaluate behaviour at various points by putting in different environmental conditions data.

Material balance formulae in the models are generally expressed using the following differential equation:

$$Dmi/dt = Qi - \Sigma jAi,j - \Sigma krecMi + \Sigma jIj,i$$

where, the left hand side of the equation is the time dependent change in chemical quantity (Mi) in a medium (I), and on the right hand side of the equation, Qi is the amount of chemical discharge into the medium, ΣjAi,j is the amount of chemical transferred into the adjacent media, ΣkrecMi is the amount of chemical disappearance by degradation in the medium, and ΣjI,j is the amount of in-flow for the adjacent media.

In general, equations which are used for the calculation of material balances are sometimes essentially empirically derived. Some of them have been widely verified by experiments, and are very reliable. On the other hand, there are some equations which are based on little data, and which are not reliable.

Data output from environmental behaviour models is in the form of displayed calculation results, or output to a printer or to floppy disks. Calculation results are shown in the form of figures or numbers. Output figures are simple and useful as a summary of calculation results. However, numeric output cannot be omitted if one wishes to discuss and utilise the model's calculation results in detail.

IV.2.4.2 Multimedia models

A. Construction

Multimedia models look at the behaviour of chemicals on a large scale. Chemical distribution and concentration in each environmental medium are evaluated from the mass balance of chemicals in the total, multi-media environment. An environment with useful, generalised character is used to make chemical behaviour in the environment understood. They are called "evaluative environments", "generic environments", or "unit worlds". Generally, evaluations of chemical behaviour in the environment is possible by just using chemical characteristic data. Multimedia environments in many models consist of the following four or six compartments – the atmosphere, water, soil, and sediment, with perhaps also suspended substances and aquatic organisms. There are other models which add further compartments, such as atmospheric particles and terrestrial organisms to the six compartments. Chemical substances are often assumed to be evenly distributed in each compartment that makes up the multi-media environment.

B. Classification and criteria

Generally multimedia models can be classified from the type of environmental processes they consider into a) mass-conserving / non-mass-conserving, b) equilibrium / non-equilibrium, and c) stationary state / non-stationary state.

a) **mass-conserving / non-mass-conserving models**

Chemicals discharged into the environment generally degrade by biological and chemical processes, and are then transported out of the system. However, mass-conserving models evaluate chemical distribution in the environment according to transport process between media without considering chemical decomposition. Therefore, chemical mass will be conserved in the environment which this model targets. These kinds of models are used at the very beginning of environmental chemical behaviour evaluation, such as when evaluating the behaviour of a chemical whose decomposition rates are unknown. On the other hand, non-mass-conserving models consider each transport, transfer, and degradation process, and chemical mass isn't conserved in the environment.

b) **equilibrium / non-equilibrium models**

Equilibrium models are models which describe transport between media as using equilibrium constants such as Henry's rule, hypothesising that thermodynamic equilibrium distribution is

achieved. On the other hand, non-equilibrium models describe transport between media, such as evaporation from water to atmosphere, or deposition from atmosphere to water, as rate processes. These models don't hypothesise distribution equilibrium, since in reality, it is rare that chemical distribution between media reaches equilibrium.

c) **stationary state / non-stationary state models**

It has already been mentioned that material, or mass balance equations expressed by evaluation of chemical behaviour in the environment can be generally expressed using the differential equation :

$$dMi/dt = Qi - \Sigma jAi,j - \Sigma krecMi + SjIj,i$$

For many general industrial chemicals, discharge into the environment is continuous, and it may be considered that a stationary, or steady state is established. Therefore, it becomes easier to gain answers because material balance formulae become simultaneous equations of the following form :

$$Qi - \Sigma jAi,j - \Sigma krecMi + \Sigma jIj,i = 0$$

Non-stationary state type models evaluate chemical behaviour and concentration with time by solving differential equations. In other words, the difference between non-stationary and stationary state models is that whether differential equations are solved or not. The processes by which material balance equations are formulated is the same. In the multimedia models, at the time of model calculation some select stationary states, some non-stationary states.

Based on these criteria, actual multi-media models are further categorised into four levels : level I, II, III, and IV. Levels I - IV of the fugacity model, which is the most popular and basic multi-media models, are equivalent to the following classifications:

Level I model categorisation may be applied to mass conserving, equilibrium and stationary state models. As mentioned above, chemical decomposition processes are not considered, and theoretical distribution of chemicals between compartments based on thermodynamic equilibrium calculated from equilibrium distribution coefficients and compartment capacities. Level I is useful for confirming which environmental media should be studied in more detail. For calculation, the following parameters are needed : chemical discharge amount into the environment, Henry's law constant, adsorption constant of soil and sediment, and bio-concentration factor. In the case of hydrophobic compounds, these distribution coefficients can be calculated from solubility and vapour pressure data.

Level II model categorisation may be applied to equilibrium and stationary state models that

are non-mass-conserving because they consider processes of transport and decomposition. The Level II model gives the same results for the environmental distribution of chemicals as Level I, because the Level II model is also an equilibrium type. Although the persistence of chemicals in the environment is evaluated, the estimates are suspected to be far from the reality. For calculation, decomposition ratio constants and discharge ratio data are needed, as well as the equilibrium constants needed for the Level I model.

Level III model categorisation may be applied to non-mass-conserving, non-equilibrium and stationary state models. Like the Level II model, Level III evaluates chemical distribution in the environment, concentration in each environmental media, and the persistence. However, transfer dynamics between compartments are also considered. For Level III non-equlibrium models, material balances between each compartment are needed. By solving these, level III models evaluate environmental behaviour much more accurately than level II models. For calculation, discharge rate data for each media are needed, as well as the same parameters required for level II. In addition, level III models require of data transfer rates between compartments, although many transfer processes are automatically estimated within the model.

Level IV model categorisation may be applied to non-mass conserving, non-equilibrium, and non-stationary state models. Level IV models can evaluate how long is needed for environmental purification after termination of chemical discharge into the environment, or how long is needed to reach stationary state in the case of continuous chemical discharge, or environmental behaviour when discharge is intermittent, eg pesticide use. The parameters required for the calculations are the same as level III model.

IV.2.4.3 Examples of multimedia models

It is difficult for users to choose which model to use from the many multimedia models available. To begin with, the reason for using the model has to be clarified. For example, when it is necessary to estimate chemical distribution in the environment, it is important to choose only Level I and II models, because less parameters are required, and the models are easy to use. However, if one must evaluate behaviour close to reality, it is important to choose Level III and IV because they have more parameters to be put in the models. Models classified into Level II should be used for general chemical behaviour in a fairly realistic environment.

In general, multimedia models are suitable for the evaluation of background concentrations and average concentrations of chemicals in environments located far from the discharge sources, and the evaluation of chemical behaviour in the wide range of environment. One must be careful with calculate results from calculations which deviate from these applications. The accuracy of the results from multimedia model relies on input parameters, especially environmental conditions

and ratio constants. Which parameter significantly affects chemical behaviour in the environment can be clarified by sensitivity analysis. As the result of sensitivity analysis, to check adequacy of the values is indispensable as for parameters which are expected to have big effects on chemical behaviour in the environment.

Here after, a few typical models will be outlined. They are all almost equivalent with respect to being based on experimental data, and because they are already developed and available, they are expected to be highly practical models. Thereafter, MNSEM is described. This model has been developed in Japan. It has an improved distinguishing feature which fits the environmental character of "generic environment" with the character of the region, and makes behaviour evaluation more accurate in the target region.

A. Fugacity models

Fugacity models are the most commonly used multimedia models. By using from Level I to IV models, one can distinguish between simple distribution equilibrium in the environment and the behaviour of easily degradable compounds in the stationery and non-stationery states. This model then becomes the basis of a number of other models. A Level III fugacity model is suitable for the stationery state evaluation of the behaviour of continuously discharged organic chemical, and the distribution, concentration, persistence, and main transfer routes of the chemicals. Level III models deal with four kinds of bulk compartment - air, water, soil and sediment. Each bulk compartment consists of sub-compartments of air, water and organic materials, and it is assumed that a distribution equilibrium is set up inside the compartment. By solving material balance equations which consider the amount of environmental discharge, diffusive or non-diffusive transfer, advection, and degradation process, the fugacity of each compartment is obtained. Thereafter, the concentration and amount of chemicals are determined from the fugacity. Necessary input data are environmental conditions, chemical / substance material, degradation rate, and discharge data.

B. Enpart (Environmental Partitioning Model)

Enpart is a fugacity model which was developed by US EPA for application to organic chemicals. It evaluates residue persistence, and the possibility of bioconcentration by comparing concentration ratio between environmental media. It is used as screening method for selection of chemicals which need detailed evaluation.

C. EEP (Environmental Exposure Potentials)

This model aims to evaluate the behaviour of new chemicals in the environment. This belongs to Level II of fugacity models, and is applied to compounds which are imported, or of which there is more than one ton per year produced in the EC countries. It evaluates distribution into environmental media and the possibility of chemical persistence, but doesn't evaluate environmental concentration. It considers only degradation by micro-organisms.

D. SIMPLESAL

This is a fugacity model which evaluates the concentrations of organic compounds and heavy metals in the stationary and non-stationary state. This has been developed in the Netherlands in order to regulate environmental discharge of existing and new organic compounds, and a screening method to conduct evaluation under various conditions. This model considers such processes as transport, transfer, and degradation in the atmosphere, water, suspended particles, aquatic living things, sediment, and soil.

E. GEOTOX

This model have been developed by US Department of Energy, and considers chemical distribution, degradation, diffusive and non-diffusive transfer processes in the atmosphere, living things, soil, surface water, and sediment. Soil is categorised into three layers : top soil, sub-soil, and under ground water. The calculated concentrations in each environmental media are correlated with inhalation rate, intake rate and absorption ratio to evaluate human body exposure. Chemical bio-concentration in terrestrial plants is given by the concentration in soil and plants / soil partition coefficient, and this is used for estimation of the amount of human body exposure. This model was developed using the environment of south east area of the United States. However, it can be applied to other areas by changing environmental conditions. In addition, this model can be applied not only when chemical discharge rate is constant, but also when the rate changes over time.

F. MNSEM

MNSEM is a model which has been developed in order to evaluate the environmental behaviour of organic chemicals in the stationary state in Japan. Chemical discharge rate is

estimated using annual production figures, usage data, and physicochemical characteristics. Based on this, the amount of human body exposure is evaluated from chemical partition, persistence, typical concentration in the Japanese environment, purification period when environmental chemical discharge stops, concentrations in various environmental media, inhalation rate, and intake rate. The environment consists of the following four media : the atmosphere, water, soil, and sediment, and each phase consists of air, water and particle compartments. Partition equilibrium is set up not between phases but between each compartment within phases, and expressed as equilibrium constants. Processes of transport, transfer, and degradation are expressed as rate constants. Precipitation, surface effusion, sedimentation of suspension particles, and soil leaching are considered. Hydroxy radical oxidation in the atmosphere, photodegradation, hydrolysis in water, and biodegradation in water system and soil are considered as degradation processes. This model will be described in detail later.

G. SMCM (Spatial Multimedia Compartment Model)

SMCM was developed by UCLA, and evaluates chemical behaviour in an environment which consists of the atmosphere, water, soil and sediment in the stationary and non-stationary states. This model is noted for its ability to evaluate vertical distribution of chemicals in soil and sediment, using equilibrium constants and rate constants in response to environmental temperature without assuming homogeneous media concentrations. Such treatment needs complex modelling calculations, but makes it possible to obtain more realistic evaluations. This model expresses chemical movement between media as mass movement coefficient and diffusion coefficient. Although these coefficients are calculated by models, necessary input parameters are almost the same as fugacity model Level III.

H. Toxscreen

Toxscreen was developed by US EPA, and evaluates the behaviour of chemicals which are released into the atmosphere, surface water, and soil, and the possibility of human exposure. Chemical behaviour in the atmosphere is evaluated using a Gaussian distribution diffusion model, and EXAMS, a water system environment model, and SESOIL, a soil model, are used to evaluate behaviour in aqueous systems and soil. Thus this model is combination of useful single-media models. The atmospheric diffusion model can be applied to all discharge sources, such as point source, linear source, or surface sources. Therefore, this model makes behaviour evaluation in small areas possible. However, a wide range of parameters necessary for the calculation.

IV.2.4.4 MNSEM2

The first version of the Multi-phase Non-Steady State Equilibrium Model (MNSEM) was released in 1987. After addition of some improvements, MNSEM145I(Version 1.4.5I) was confirmed as the model which can be used for the evaluation of the amount of indirect human exposure at the OECD Workshop on the Application of Simple Models for Environmental Exposure Assessment (Berlin, Germany) in 1992. MNSEM2 (Version 2.0) is the program which has supplanted the MS-DOS based MNSEM145I onto Windows, and has been further improved by adding new knowledge about material movement between environmental media, and calculated exposure doses which have been reported since 1993. The program of this model is available from database of the National Institute for Environmental Studies through w-chemdb@nies.go.jp.

MNSEM2 basically follows the concept of the former version (MNSEM145I). However, some of the default values and some of the formulae which are used for the calculation of material movement between environmental media and exposure doses were revised based on more recent research results.

A. Input parameters

Weather conditions and the environmental, chemical substances, and discharge feature which are needed for MNSEM2 will be described. The default values of these characteristics are not always suitable for every evaluation. It is recommended that users should wherever possible use values which are suitable for their own evaluation.

a) Weather conditions and the environment

Table IV-2-1 Weather conditions and environmental feature for calculation

Parameters	abbreviation	units	default values
Environmental area for evaluation	SUA	m^2	1×10^{10}
Temperature	TEMPK	°C	20
land area ratio	LLS	-	0.8
wind velocity	AFR	m/sec	3.2
Precipitation	TRF	mm/year	1500
Atmospheric altitude	DEPA	m	200
Concentration of aerosol	CAER	mg/m^3	0.03 [1)]
Density of aerosol	DENAER	kg/m^3	1500 [1)]
Diameter of aerosol	DAER	μm	10
OH radical concentration	OHC	$molecule/cm^3$	1×10^6 [2)]
depth of surface water	DEPW	m	10
Suspended particles	CWSS	mg/L	50
Aquatic organisms	CWB	mg/L	5
ratio of organic carbon content to amount of suspended particles	OCSS	-	0.06
rate constant of surface water advection rate	KWAD	1/day	0.1
sedimentation velocity of suspended particles	KSV	M/day	0.5
decay ratio of photodegradation in water	PAIW	-	0.1
surface water pH	PHW	-	7.0
aquatic microorganisms	AMWW	cell/L	1×10^5
depth of soil	DEPSO	m	0.20
air volume ratio in soil	SOAF	-	0.2 [3)]
water volume ratio in soil	SOWF	-	0.3 [3)]
organic carbon content ratio in soil particles	OCSOS	-	0.04
density of soil particles	DENSOS	kg/L	1.5
pH of water in soil	PHSO	-	7.0
evaporation ratio of water in soil	ETP	-	0.35
soil erosion rate	ERS	m/year	0.0002 [1)]
aquatic microorganisms in soil	AMSOW	cell/L	1×10^5
microorganisms in soil particles	AMSOS	cell/kg	1×10^8
depth of sediment	DEPSE	m	0.05
water volume ratio in sediment	POSE	-	0.75
organic carbon content ratio in sediment particles	OCSES	-	0.06
density of sediment particles	DENSES	kg/L	2.0
pH of sediment water	PHSE	-	7.0
aquatic microorganisms in sediment	AMSEW	cell/L	1×10^5
microorganisms in sediment	AMSES	cell/kg	1×10^8

b) Chemical compound parameters

Table IV-2-2 Chemical compound parameters for calculation

Parameters	abbreviation	units	default values
molecular weight	MW	g/mol	119.4
melting point	TEMPMP	°C	-25
water solubility	WS	mg/L	8000
vapour pressure	Pa	Pa	21332
log Kow	KOW	-	1.97
absorption constant of organic carbons	KOC	L/kg	280
fish bioconcentration factor	BCF	L/kg	15
reaction with atmospheric OH radical	KOH	cm^3/mole/sed	9.7×10^{-14}
photodegradation in water	KPHT	1/day	0
Hydrolysis	KHY	L/mole/dec	0
Biodegradation	KBIO	L/cell/day	0

Note : The default compound is chloroform, and chloroform's characteristics, equilibrium constants and velocity constants are set up.

c) Discharge parameters

Table IV-2-3 Discharge parameters for calculation

Parameters	abbreviation	units	default values
annual production	PM	ton/y	36000
use ratio (closed system utilisation)	FCU	-	0.88
Total Japanese production / evaluation area	EVAI	-	production amount in whole Japan
discharge coefficient (production, atmosphere)	EPRA	-	0.10
discharge coefficient (production, surface water)	EPRW	-	0.00
discharge coefficient (production, soil)	EPRS	-	0.00
discharge coefficient (closed system, atmosphere)	ECUA	-	0.20
discharge coefficient (closed system, surface water)	ECUW	-	0.00
discharge coefficient (closed system, soil)	ECUS	-	0.00
discharge coefficient (open system, atmosphere)	EOUA	-	0.95
discharge coefficient (open system, surface water)	EOUW	-	0.05
discharge coefficient (open system, soil)	EOUS	-	0
background concentration of inflow atmosphere	CBGA	mg/m^3	0.00
background concentration of inflow surface water	CBGW	mg/L	0.00

Note : Default values are those of chloroform.

d) Intake parameters

Data covering human intake parameters are necessary for the calculation. The National Diet Survey classifies vegetables as yellow-green vegetables (carrot, spinach, green pepper, tomato, etc.) and other vegetables (radish, onion, cabbage, cucumber, Chinese cabbage, salted leaf vegetables / radish pickles). However, the model parameters of leaf vegetables and root vegetables don't correspond to these. Therefore, the sum of 300 g of yellow-green vegetables and the other vegetables are assumed as leaf vegetables : root vegetables = 2 : 1 for default values.

Table IV-2-4 Intake parameters for calculation

Parameters	abbreviation	units	default values
body weight (human)	BW	Kg	60
air inhalation rate (human)	INTKA	m^3/day	20
bio-availability from inhalation	BIOAV	-	0.75 [6]
intake of drinking water (human)	INTKDW	L/day	2.0
purification ratio of drinking water source	PFW	-	0
intake of marine products (human)	INTKF	g/day	100 [5]
intake of leaf vegetables and fruits (human)	INTKLC	g/day	320 [5]
intake of root vegetables (human)	INTKRC	g/day	100 [5]
intake of meat products (human)	INTKME	g/day	80 [5]
intake of dairy products (human)	INTKMI	g/day	130 [5]
intake of soil (human, unintentional)	INTKS	g/day	0.05 [7]
air inhalation rate (livestock)	CTKINHL	m^3/day	122 [6]
pasture (dairy cattle, dry)	CTLGRASSL	kg/day	16 [8]
pasture (beef cattle, dry)	CTLGRASSN	kg/day	8 [8]
soil (livestock, unintentional, dry)	CTLSOIL	kg/day	0.41 [6]

B. Detailed description of the models

a) Material movement in the atmosphere

The atmosphere consists of the following compartments : air, rain water, and aerosol. MNSEM treats chemicals distributed into the compartments of air, rain water, and aerosol in the atmospheric phase as chemicals in the gas phase, dissolved phase, and adsorbed phase, respectively.

(1) Mass distribution ratio

The amount (mass) of material in the gas phase (MMA) and the mass of adsorbed material (MAP) are related by the following formula :

$$MAAP = MAA + MAP$$
$$MAA = MAAP \cdot (1 - FP)$$
$$MAP = MAAP \cdot FP$$

where MAAP is the sum of the gaseous and adsorbed phase material, FP is the ratio of the mass of adsorbed material to the sum of the gas phase and adsorbed phase material calculated by the following Junge formula :

$$FP = CJ \cdot SP / (VPL + CJ \cdot SP)$$

where CJ is constant of the Junge expression, SP is surface area of the aerosol, VPL is vapour pressure of the liquid phase (the vapour pressure of supercool liquid phase is used when the melting point of the chemical is higher than environmental temperature.

The ratio (RRT) of total chemical concentration in the gas and adsorbed phase (CAAP) and chemical concentration in rain water (CAW) is determined by the following formula :

$$RRT = CAW / CAAP = (1 - FP) / HENRY + 2 \times 10^5 \cdot FP$$

where, HENRY is Henry's law constant, and 2×10^5 is the trap ratio of aerosol.

When the total chemical volume of the air and aerosol compartment is given as VOLAAP, and the volume of rain water compartment, VOLAAP, then the amount of chemical in the rain water compartment is determined by the following equation :

$$MAW = RRT \cdot MAAP \cdot VOLAW / VOLAAP$$

The total chemical mass in the atmosphere (MSA) is determined by following formula :

$$MSA = MAA + MAP + MAW = (1 + RRT \cdot VOLAW / VOLAAP) \cdot MAAP$$

From the above formula, the chemical mass distribution ratio in air, aerosol, and rain water in the atmospheric phase (FAA, FAP, FAW respectively) can be obtained in the following manner :

$$FAA = (1 - FP) / (1 + RRT \cdot VOLAW / VOLAAP)$$
$$FAP = FP / (1 + RRT \cdot VOLAW / VOLAAP)$$
$$FAW = RRT \cdot VOLAW / VOLAAP / (1 + RRT \cdot VOLAW / VOLAAP)$$

The volume of the rain water compartment is determined by :

$$VOLAW = TRF \cdot SUA \cdot RTRF / 1000 / 365$$

where TRF is annual precipitation (mm/year), SUA is total contact area of the surface water and soil compartment, and RTRF is the standing time of the rain water in the atmosphere. A rain fall rate of 6.5 m/s is adopted, and corresponds to typical rain drop diameter of 1000 μm. Therefore, the first kinetic constant of rain fall (KARF, 1/day) is determined by the following equation, with the reciprocal number being the standing time of the rain water (RTRF).

$$KARF = 24 \times 3600 \times 6.5 / DEPA$$
$$RTRF = 1 / KARF$$

Where, DEPA is altitude of the atmospheric phase.

If the volume of the atmospheric phase (VOLA) is given by

$$VOLA = SUA \cdot SEPA$$

then VOLAAP is determined by the following formula :

$$VOLAAP = VOLA - VOLAW$$

When default values of 30 μg/m^3 for CAER and 1.5 g/cm^3 for DENAER are used, the aerosol volume ratio occupied in VOLAAP is 2×10^{-11}.

(2) Kinetic constant

The kinetic constant of advection (KAAD) is determined from the average wind velocity (AFR) by the following formula :

$$KAAD = 24 \times 3600 \times AFR / (SUA)^{1/2}$$

The kinetic constant of atmospheric dispersion (KAU) is determined by the Thornthwaite-Holzmann formula. It is known that this formula gives appropriate results when the atmospheric stability is either weakly stable, intermediate, or has weak instability where the Richardson number is less than 0.01.

$$KAU = 24 \times 3600 \times 0.412 \cdot AFR / \{\log (DEPA / 0.1)\} / DEPA$$

The kinetic constant of decomposition in the atmosphere (KAT) is determined from the following formula which explicitly considers oxidation by OH radical :

$$KAT = 24 \times 3600 \times KOH \cdot OHC$$

Where, KOH is kinetic constant of reaction with OH radical (cm^3/molecule/sec), and OHC is concentration of OH radical in the atmosphere (molecule/cm^3).

The kinetic constants against diffusive transfer from the atmosphere to surface water phase (KAW) is determined by using the following mass movement coefficient (unit : m/day).

[air phase mass movement coefficient] $KG = 24 \times 36 \times (0.3 + 0.2 \times AFR) \cdot (18/MW)^{0.4355}$
[liquid phase mass movement coefficient] $KL = 24 \times 36 \times (0.004 + 0.00004 \times AFR) \cdot (32 / MW)^{0.4047}$
[air / liquid boundary mass movement coefficient] $KGL = 1 / (1 / KL + 1 / HENRY / KG)$

KAW is determined by the following formula :

$$KAW = KGL / HENRY / (DEPA / LLW)$$

Where, LLW is the ratio of surface water to total surface area (SUA).

The kinetic constants of diffusive transfer from the atmospheric phase to soil phase (KAS) is determined by the double thin layer theory using the following mass movement coefficient (unit : m/day) :

[air in soil mass movement coefficient] $KASLSA = 24 \times 3600 \times 0.00000556$
[water in soil mass movement coefficient] $KASLSW = 24 \times 3600 \times 0.000000000556$

KAS is determined by the following formula :

$$KAS = (KG \cdot KASLSA + KG \cdot KASLSW / HENRY) / (KG + KASLSA + KASLSW / HENRY) / (DEP / LLS)$$

Where, LLS is the ratio of soil to the total surface area (SUA).

The aerosol deposition kinetic constant (KAEF) is determined from the following formula :

$$KAEF = 2 \times (DAER / 1000000)^2 \cdot DENAER \cdot 9.8 / 9 / 0.000015 / DENAIR \cdot 3600 \times 24 / DEPA$$

Where, DAER is particle diameter (m), DENAER is particle density (kg/m^3), and DENAIR is air density (1.293 kg/m^3).

(3) Average standing time

Average standing (residence) time in the atmosphere (TA) is determined by the following formula combining mass distribution ratio and kinetic constants.

$$TA = 1 / (KAAD + (KAT + KAW + KAS + KAU) \cdot FAA + KARF \cdot FAW + KAEF \cdot FAP)$$

(4) Disappearance contribution ratio

Contribution of advection, dispersion, decomposition, diffusive transport (surface water and soil), precipitation and aerosol deposition to chemical disappearance in the atmosphere is determined by the following formulae :

[advection] $COAAD = 100 \times KAAD \cdot TA$

[dispersion] $COAU = 100 \times KAU \cdot FAA \cdot TA$

[decomposition] $COAT = 100 \times KAT \cdot FAA \cdot TA$

[diffusion (water)] $COAS = 100 \times KAS \cdot FAA \cdot TA$

[diffusion (soil)] $COAS = 100 \times KAS \cdot FAA \cdot TA$

[precipitation] $COARF = 100 \times KARF \cdot FAW \cdot TA$

[particle deposition] $COAEF = 100 \times KAEF \cdot FAP \cdot TA$

b) Material movement in soil phase

The soil phase consists of three compartments : air, water and particles. MNSEM deals with chemicals which are distributed into soil air, water, and particle compartments in the same way as chemicals in gas phase, dissolved phase, and adsorption phase respectively.

(1) Mass distribution ratio

Chemical mass distribution ratios in air, water, and particles in soil phase (FSOA, FSOW, FSOS) are determined by the following formulae :

$$FSOA = HENRY \cdot SOAF / (HENRY \cdot SOAF + SOWF + KOC \cdot OCSOS \cdot (1 - SOAF - SOWF) \cdot DENSOS)$$

$$FSOW = SOWF / (HENRY \cdot SOAF + SOWF + KOC \cdot OCSOS \cdot (1 - SOAF - SOWF) \cdot DENSOS)$$

$$FSOS = KOC \cdot OCSOS \cdot (1 - SOAF - SOWF) \cdot DENSOS / (HENRY \cdot SOAF + SOWF + KOC \cdot OCSOS \cdot (1 - SOAF - SOWF) \cdot DENSOS)$$

Where, SOAF and SOWF are the soil : air volume and the soil : water volume ratios, respectively, and OCSOS and DENSOS are the organic carbon content, and density of soil particles respectively. KOC is organic carbon adsorption constants of the chemicals.

(2) Kinetic constants

The kinetic constant for evaporation from soil phase to the atmosphere is determined by using mass transfer coefficient mentioned above.

$$KSA = 1 / (1 / KG / HENRY + 1 / (KASLSA \cdot HENRY + KASLSW))/(SOAF \cdot HENRY + SOWF + (1 - SOAF - SOWF) \cdot KOC \cdot OCSOS \cdot DENSOS) / DEPSO$$

Where, DEPSO is depth of soil.

Degradation in soil, microbial degradation and hydrolysis in soil water (dissolved state) and microbial degradation in soil particles (adsorption state) are considered.

$$KSOW = KBI0 \cdot AMSOW + 24 \times 3600 \times KHY \times 10\ PHSO - 14$$

$$KSOS = KBIO \cdot AMSOS$$

Therefore, the kinetic constant of degradation (KSOT) is determined by the following formula :

$$KSOT = FSOW \cdot KSOW + FSOS \cdot KSOS$$

Surface effusion and leaching from soil accompanied by precipitation contribute to the disappearance of dissolved substances in soil. The kinetic constants of effusion and leaching are expressed as the following :

[surface effusion] $KSRO = SRF / 1000 / DEPSO / SOWF / 365$

[leaching] $KSLE = RLE / 1000 / DEPSO / SOWF / 365$

Where, SRF and RLE are the amount of water effusing and leaching in both mm/year, respectively. RLE is determined as the product of the penetration coefficient (KPED, mm/day) and the number of days of precipitation.

$$KPED = 0.01 / 96 \cdot ADS^2 \times 980.7 / 0.010038 \times 10 \times 24 \times 3600$$

ADS is average diameter of soil particles (cm).

The number of days of precipitation is considered to be 100 days, or the number of days which have more than 1 mm/day of rainfall. Therefore, SRF is determined by the following formula :

$$SRF = TRF \cdot (1 - ETP) - RLE$$

Where, erosion by precipitation and by wind contribute to the disappearance of substances adsorbed onto soil, the kinetic constants of rainfall and wind erosion are expressed as the following :

[erosion] $KSER = ERS / 365 / DEPSO / (1 - SOAF - SOWF)$

[lofting] $KSRSUP = CAER / 1000 \cdot VOLA \cdot KAEF / VOLSO / (1 - SOAF - SOWF) / DENSOS / 1000000$

Where, ERS is soil erosion rate (m/year) and CAER is atmospheric aerosol concentration (mg/m^3). It is clear from the calculation of KSRSUP that soil particles lofted by wind into the atmosphere is balanced by particle adsorption from the atmosphere.

(3) Average residence time

The average standing time in soil (TS) is determined by the following formula using mass distribution ratio and kinetic constants :

$$TS = 1 / (KSA + FSOW \cdot (KSOW + KSRO + KSLE) + FSOS \cdot (KSOS + KSRSUP + KSER))$$

(4) Disappearance contribution ratio

The contribution of evaporation, degradation, surface effusion, leaching, erosion, and lofting into the atmosphere to the disappearance of chemicals in soil are determined by the following formulae :

[evaporation] $COSA = 100 \times KAAD \cdot TA$

[degradation] $COAT = 100 \times KAT \cdot FAA \cdot TA$

[surface effusion] $COAU = 100 \times KAU \cdot FAA \cdot TA$

[leaching] $COAS = 100 \times KAS \cdot FAA \cdot TA$

[erosion] $COAS = 100 \times KAS \cdot FAA \cdot TA$

[lofting] **COARF = 100 × KARF·FAW·TA**

c) Material movement in surface water phase

The water or aqueous phase consists of three compartments : water, suspended particles, and aquatic organisms. MNSEM treats chemicals which are distributed into water, on suspended particles and in aquatic organisms as chemicals in the dissolved phase, adosrption phase and biological phase respectively.

(1) Mass distribution ratio

Mass distribution ratios of chemicals in water, suspended particles and aquatic organisms in the water phase (FSS, FWSS, FWB) are determined by the following formulae :

$$FWW = VOLW / (VOLW + KOC \cdot OCSS \cdot WSS + BCF \cdot WB)$$

$$FWSS = KOC \cdot OCSS \cdot WSS / (VOLW + KOC \cdot OCSS \cdot WSS + BCF \cdot WB)$$

$$FWB = BCF \cdot WB / (VOLW + KOC \cdot OCSS \cdot WSS + BCF \cdot WB)$$

Where, WSS and WB are the amount of suspended material (ton) and aquatic oranisms (ton) in the surface water phase. The concentration of suspended materials (CWSS, mg/L) and concentration of aquatic organisms (CWB, mg/L) are determined by the following formulae :

$$WSS = CWSS \cdot VOLW / 1000000$$

$$WB = CWB \cdot VOLW / 1000000$$

(2) Kinetic constants

Aqueous phase degradation, photodegradation, hydrolysis and microbial degradation in the dissolved phase must be considered. The kinetic constants of degradation (KWT) are determined by the following formula :

$$KWT = (KPHT \cdot PAIW + KBIO \cdot AMWW + KHV \times 10\ PHW - 14 \cdot 24 \times 3600)$$

Where KPHT is the kinetic constants of photodegradation in water (1/day), and PAIW is decay ratio of photodegradation by light scattering. KBIO and AMWW are the biodegradation kinetic constants (L/cell/day) and microbiological amount in surface water (cell/L), respectively. And KHY and PHW are the hydrolysis kinetic constants (L/mole/sec) and pH of surface water, respectively.

The kinetic constants of evaporation from the water phase to the atmospheric phase (KWA) are

determined by using air/liquid boundary mass transfer coefficients (KGL), and :

$$KWA = KGL / DEPW$$

Where, DEPSW is depth of the surface water phase.

The kinetic constants of diffusion transfer from the water phase to the sediment phase are determined by the double thin layer theory using the following mass transfer coefficients (unit: m/day) :

[surface water side mass transfer coefficients] $KWSW = 24 \times 3600 \times 0.000002778$
[sediment water side mass transfer coefficients] $KWSS = 24 \times 3600 \times 0.00000002778$

KWSE is determined by the following formula :

$$KWSE = KWSW \cdot KWSS / (KWSW + KWSS) / DEPW$$

The kinetic constants of transfer to the sediment phase accompanied by sedimentation of suspended particles in water phase (KWSV) are determined by the following formula :

$$KWSV = KSV / DEPW$$

Where, KSV is sedimentation rate of suspended particles (m/day).

(3) Average residence time

The average residence time in surface water (TW) is calculated by the following formula using mass distribution ratio and kinetic constants. Chemical disappearance by metabolism inside of aquatic organisms is not considered.

$$TW = 1 / (FWW \cdot (KWAD + KWT + KWA + KWSE) + FWSS \cdot (KWAD + KWSV))$$

(4) Disappearance contribution ratio

The contribution of advection, degradation, evaporation, diffusive transfer into sediment and sedimentation to chemical disappearance in surface water are determined by the following formulae :

[advection] $COWAD = 100 \times (FWW + FWSS) \cdot KWAD \cdot TW$

[degradation] $COWT = 100 \times FWW \cdot KWT \cdot TW$

[evaporation] $COWA = 100 \times FWW \cdot KWA \cdot TW$

[sediment diffusion transfer] $COWSE = 100 \times FWW \cdot KWSE \cdot TW$

[sedimentation] $COWSV = 100 \times FWSS \cdot KWSV \cdot TW$

Where, KWAD is the advection kinetic constants of surface water (1/day).

d) Material movement in sediment phase

The sediment phase consists of two compartments : interstitial pore water and particles. MNSEM treats chemicals which are distributed in interstitial pore water and particles as being dissolved and adsorbed chemicals, respectively.

(1) Mass distribution ratio

Mass distribution ratio of chemicals in interstitial pore water and particles in the sediment phase (FSEW, FSES) are determined by the following formulae :

$$FSEW = POSE / (POSE + KOC \cdot OCSES \cdot (1\text{-}POSE) \cdot DESES)$$

$$FSES = KOC \cdot OCSES \cdot (1-POSE) \cdot DENSES / (POSE + KOC \cdot OCSES \cdot (1-POSE) \cdot DENSES)$$

(2) Kinetic constants

Degradation in the sediment phase, hydrolysis and microbial degradation in the dissolved state and microbial degradation in adsorption state must be considered. The kinetic constants of degradation in dissolved state and adsorption state (KSET and KSEST) are determined by the following formulae :

$$KSEWT = (KBIO \cdot AMSEW + KHY \times 10^{PHSE-14} \cdot 24 \times 3600)$$

$$KSEST = KBIO \cdot AMSES$$

Where, AMSEW and AMSES are microbiological amount in sediment interstitial pore water and sediment particles (cell/L or cell/kg). And PHSE is pH of sediment interstitial pore water.

Therefore, the kinetic constant of degradation (KSET) is determined by the following formula:

$$KSET = FSEW \cdot KSEWT + FSES \cdot KSEST$$

The kinetic constant of diffusive transfer from the sediment phase to surface water phase

(KSEW) is determined by the double thin layer theory as mentioned above :

$$KSEW = KWSW \cdot KWSS / (KWSW + KWSS) / (DEPSE \cdot POSE)$$

The kinetic constants of particle re-suspension from the sediment phase to surface water phase (KSERS) is determined by the following formula :

$$KSERS = 0.25 \times KWSV \cdot WSS / VOLSE / (1 - POSE) / SENSES$$

As is clear from the above formula, particle re-suspension from sediment to surface water is assumed to be 25 % of the amount of particle sedimentation.

(3) Average residence time

The average residence time in the sediment phase (TSE) is determined by the following formula involving mass distribution ratio and kinetic constants :

$$TSE = 1 / (FSEW \cdot (KSEW + KSEWT) + FSES \cdot (KSEST + KSERS))$$

(4) Disappearance contribution ratio

The contribution of degradation, diffusion transfer into surface water phase, and re-suspension of sediment particles to chemical disappearance in sediment phase is determined by the following formulae :

[degradation] $COSET = 100 \times KSET \cdot TSE$

[diffusion into water phase] $COSEW = 100 \times FSEW \cdot KSEW \cdot TSE$

[resuspension] $COSERS = 100 \times FSES \cdot KSERS \cdot TSE$

e) Material balance in the environment

Chemical material balances in an environment consisting of four kinds of phases - the atmosphere, surface water, soil, and sediment - is expressed by the following differential equations :

[atmospheric phase] $dMSA/dt = TEMA + A(1,1) \cdot MSA + A(1,2) \cdot MSW + A(1,3) \cdot MSSO + A(1,4) \cdot MSSE$

[surface water phase] $dMSW/dt = TEMW + A(2,1) \cdot MSA + A(2,2) \cdot MSW + A(2,3) \cdot MSSO + A(2,4) \cdot MSSE$

[soil phase] $dMSSO/dt = TEMS + A(3,1) \cdot MSA + A(3,2) \cdot MSW + A(3,3) \cdot MSSO + A(3,4) \cdot MSSE$

[sediment phase] $dMSSE/dt = A(4,1) \cdot MSA + A(4,2) \cdot MSW + A(4,3) \cdot MSSO + A(4,4) \cdot MSSE$

Where, MSA, MSW, MSSO, and MSSE are the amounts of chemical material in the atmospheric, surface water, soil, and sediment phases, respectively. And TENA, TEMW, and TEM are the amounts of chemical discharged into the atmospheric, surface water, and soil phases, respectively. Coefficients A(1,1) - A(4.4) can be expressed by using the mass distribution ratio, kinetic constants, and average residence time in each phase using the following:

$A(1,1) = -1 / TA$

$A(1,2) = KWA \cdot FWW$

$A(1,3) = KSA + FSOS \cdot KSRSUP$

$A(1,4) = 0$

$A(2,1) = KAW \cdot FAA + LLW \cdot (KARF \cdot FAW + KAFE \cdot FAP)$

$A(2,2) = -1 / TW$

$A(2,3) = KSROP \cdot FSOW + FSOS \cdot KSERS$

$A(2,4) = KSEW \cdot FSEW + FSES \cdot KSERS$

$A(3,1) = KAS \cdot FAA + LLS \cdot (KARD \cdot FAW + KAEF \cdot FAP)$

$A(3,2) = 0$

$A(3,3) = -1 / TS$

$A(3,4) = 0$

$A(4,1) = 0$

$A(4,2) = KWSE \cdot FWW + KWSV \cdot FWSS$

$A(4,3) = 0$

$A(4,4) = -1 / TSE$

MNSEM2 assumes that chemical discharge into the environment is continuous and constant and the amount of chemical material in the environment (concentration) is in a stationary, or steady state. That is, it is assumed that dMSA/dt = dMSW/dt = dMSSO/dt = dMSSE/dt = 0. Therefore, the amount of chemical material in each phase in the environment is obtained by solving the following simultaneous equations :

$A(1,1) \cdot MSA + A(1,2) \cdot MSW + A(1,3) \cdot MSSO + A(1,4) \cdot MSSE = TEMA$

$A(2,1) \cdot MSA + A(2,2) \cdot MSW + A(2,3) \cdot MSSO + A(2,4) \cdot MSSE = TEMW$

$A(3,1) \cdot MSA + A(3,2) \cdot MSW + A(3,3) \cdot MSSO + A(3,4) \cdot MSSE = TEMS$

$A(4,1) \cdot MSA + A(4,2) \cdot MSW + A(4,3) \cdot MSSO + A(4,4) \cdot MSSE = 0$

f) Calculation of the amount of exposure

Routes of indirect exposure to humans via the environment include exposure from inhalation of air, drinking of water, eating marine, meat and dairy products, eating root and leaf vegetables, and unintentional intake of soil.

(1) Exposure through inhaled air

The amount of exposure via inhaled air is determined by the following formula :

$$EXPA = CACON \cdot INTKA \cdot BIOAV / BW$$

Where, CACON is the concentration in the atmospheric environment (mg/m^3), and is calculated by the equation as mentioned above. INTKA is daily air inhalation rate (m^3/day), BIOAV is biological use ratio against air inhalation, and BW is human body weight. Default values of these exposure factors are shown in **Table IV-2-4**.

(2) Exposure through ingestion of drinking water

The amount of chemical exposure associated with ingestion of drinking water (EXPDW, mg/kg/day) is determined by the following formula :

$$EXPDW = CW \cdot (1 - PFW) \cdot INTKDW / BW$$

Where, CW is the chemical concentration in surface water calculated by the above formula. PFW is purification ratio of drinking water source, and INTKDW is the daily intake of drinking water (L/day). Default values for these exposure factors are shown in **Table IV-2-4**.

(3) Exposure through ingestion of marine products

The amount of chemical exposure associated with ingestion of marine products (EXPF, mg/kg/day) is determined by the following formula :

$$EXPF = CFISH \cdot INTKF / 1000 / BW$$

Where, CFISH is the chemical concentration in fish in surface water phase (mg/kg) determined by the following formula :

$$CFISH = 1000000 \times CW \cdot FWB / CWB$$

INTKF is the daily intake of marine products (g/day). Default values for these exposure

factors are shown in **Table IV-2-4**.

(4) Exposure through ingestion of meat products

The amount of chemical exposure associated with meat products intake (EXPME, mg/kg/day) is determined by the following formula :

$$EXPME = CMEAT \cdot INTKME / 1000 / BW$$

Where, CMEAT, the chemical concentration in meat of livestock (mg/kg) is calculated by the following formula, and INTKME is daily intake of meat products (g/day). Default values for these exposure factors are shown in **Table IV-2-4**.

$$CMEAT = BCFMEAT \cdot (C\_grass \cdot CONWD \cdot CTLGRASSN + CA \cdot CTLINHL + CSOCON \cdot CTLSOIL \cdot CONVSOIL)$$

BCFMEAT is the biotransfer coefficient into meat products (day/kg) determined from the logarithm value of octanol/water partition coefficient (log Kow : LOGKOW).

$$BCFMEAT = 10^{-7.6 + LOGKOW}$$

Also C_grass is the chemical concentration in pasture, and this is assumed to be equal to the concentration in leaf vegetables (see later). CONWD and CTLGRASSN are the conversion coefficient from pasture wet weight to dry weight, and pasture intake by beef cattle, respectively. Since CTLGRASSN is daily pasture intake expressed as dry weight of grass, C_grass expressed on a wet weight basis is converted into a dry weight concentration by using CONWD. CA and CTLINHL are chemical concentrations in the atmosphere and the air inhalation rate of livestock, respectively. CSOCON, CONVSOIL, and CTLSOIL are chemical concentrations in the soil (mg/kg), the amount of dry soil ingested by livestock, and a coefficient representing the conversion from dry weight soil to wet weight, respectively. As CTLSOIL is dry weight intake amount, it is converted by using CONVSOIL into a wet weight intake.

(5) Exposure resulting from ingestion of dairy products

The amount of chemical exposure associated with ingestion of dairy products (EXPMI, mg/kg/day) is determined by the following formula :

$$EXPMI = CMILK \cdot INTKMI / 1000 / BW$$

Where, CMILK is chemical concentration in cow milk (mg/kg) as determined by the following formula, and INTKMI is daily intake of dairy products (g/day). Default values for these exposure factors are shown in **Table IV-2-4**.

$$CMILK = BCFMILK \cdot (C\_grass \cdot CONWD \cdot CTLGRASSL + CA \cdot CTLINHL + CSOCON \cdot CTLSOIL \cdot CONVSOIL)$$

BCFMILK is biotransfer coefficient into cow milk (day/kg), which is determined from the logarithm value of octanol/water partition coefficient (log Kow : LOGKOW).

$$BCFMILK = 10^{-8.1 + LOGKOW}$$

Also CTLGRASSL is the amount of pasture eaten by dairy cows.

(6) Exposure resulting from ingestion of root vegetables

The amount of chemical exposure associated with ingestion of root vegetables (EXPRC, mg/kg/day) is determined by the following formula :

$$EXPRC = C\_rootplant \cdot INTKRC / 1000 / BW$$

Where, C_rootplant is the chemical concentration in root vegetables (mg/kg) calculated by the following formula, and INTKRC is daily intake of root vegetables (mg/kg). Default values for these exposure factors are shown in **Table IV-2-4**.

$$C\_rootplant = BCFROOT \cdot CSOCON$$

BCFROOT is the magnification of chemical concentration in the root vegetables resulting from soil, and determined by the following formula :

$$BCFROOT = (0.82 + 10^{(0.77 \cdot LOGKOW - 1.52)}) / 1000 \cdot BDCON / KSOIL\_WATER$$

Where, BDCON is soil bulk density per kg/m^3. KSOIL_WATER is the soil/water partition coefficient, and determined by the following formula :

$$KSOIL\_WATER = SOWF + SOSF \cdot KDCON \cdot DENSOSCON$$

Where, KDCON is soil adsorption coefficient per m^3/kg, and DENSOSCON is soil particle density per kg/m^3.

(7) Exposure resulting from ingestion of leaf vegetables

The amount of chemical exposure associated with ingestion of leaf vegetables and fruit (EXPLC, mg/kg/day) is determined by the following formula :

$$EXPLC = C\_stemplant \cdot INTKLC / 1000 / BW$$

Where, C_stemplant is the chemical concentration in the leaf vegetables and which is calculated by the following formula, and INTKLC is daily intake of leaf vegetables (g/day). Default values for these exposure factors are shown in **Table IV-2-4**.

$$C\_stemplant = BCFSTEM \cdot CSOCON + BCFA\_PLANT \cdot CA$$

BCFSTEM is the magnification of chemical concentration in leaf vegetables via soil, and which is calculated by the following formula, and BCFA_PLANT is the chemical concentration magnification of leaf vegetables via the atmosphere.

$$BCFSTEM = TSCF \cdot SCF \cdot BDCON / KSOIL\_WATER$$

Where, TSCF, the partition coefficient between the water flowing in vascular system of the plant and soil water, and SCF, the concentration magnification in stem, are determined by the following formulae :

$$TSCF = 0.784 \cdot EXP \{- (LOGKOW - 1.78)^2 / 2.44\}$$

$$SCF = (0.82 + 10^{0.95 \cdot LOGKOW - 2.05}) / 1000$$

BCFA_PLANT is the magnification of chemical concentration in the leaf vegetables via the atmosphere, and is determined by the following formula :

$$BCFA\_PLANT = FAP \cdot Kaer\_plnt + FAA \cdot Kgas\_plnt$$

Where, Kgas_plnt is bioconcentration of gaseous substances in the atmosphere by plant leaves and stems, and is determined by the following formula using air volume ratio (FPA, v/v), water volume ratio (FPW, v/v), and lipid volume ratio (FPLPD, v/v) in plant.

$$Kgas\_plnt = (FPA + (FPW + FPLPD \times 10\ LOGKOW) / HENRY) / BDPLANTCON$$

Kaer_plnt, the concentration of substances in adsorption phase in the atmosphere into leaves and stems, is 3300.

(8) Exposure resulting from unintentional soil

The amount of chemical exposure associated with ingestion unintentional of soil (EXPSO, mg/kg/day) is determined by the following formula :

$$EXPSO = CSO \cdot INTKS / DENSOS / 1000 / BW$$

Where, INTKS is the daily intake of soil (g/day).

(9) Amount of total exposure

The total exposure to chemicals associated with inhalation of air, intake of drinking water, marine products, meat products, dairy products, root vegetables, leaf vegetables, and unintentional soil (THDOSE, mg/kg/day) is determined by the following formula :

$$THEDOSE = EXPA + EXPDW + EXPF + EXPME + EXPMI + EXPRC + EXPLC + EXPSO$$

IV.3 Risk assessment method

The results of environmental monitoring produce extremely important information which is used to judge the level of exposure to a chemical or chemicals by humans and other organism, and the effects of that exposure. Methods of evaluating the affect of chemicals on environmental organisms are still being developed, but a method which expresses the size of chemical effects on human health as the probability of determining the kinds of possible health effects has almost been established, and is used as an index and for setting guidelines. This risk assessment plays an important role in practically and efficiently taking countermeasures to reduce risk, and to give scientific grounds used control harmful effects to less than tolerable level.

Environmental exposure to chemicals is undoubtedly smaller than direct exposure in the working environment, such as chemical or goods production and processing areas. However, since possible environmentally influenced health risks generally do not include acute toxicity, chronic toxicity effects, such as carcinogenicity, must be considered. Therefore, risk assessment using the results of environmental monitoring is generally regarded as a life-time risk evaluation based on assumptions of continuous human exposure.

This chapter illustrates the concept of risk assessment in a step by step manner, using the risk assessment of dioxin exposure in Japan as a concrete example. In addition, since a wide range of information is needed to conduct chemical risk assessment, such as social information on use patterns, purpose, production volumes etc., physicochemical information such as solubility, character of degradation, accumulation, volatilisation, solubility, and adsorption, movement in the environment, and biological information such as toxicity, bioeffects, movement in the body, bioinfluences etc., this chapter lists sources for such information.

IV.3.1 Structure and concept of risk assessment

The word “risk” as originally used has a meaning of “probability of damage or hazard”. However, the meaning of “risk” when “risk” is used in environmental “risk assessment” indicates “any danger or dangerous things which may have a harmful influence on humans or the environment”, and this definition includes not only the “probability of danger happening and the size of its influence” but also “how society evaluates it”

An assessment of chemical risk to humans should be based on the best scientific knowledge and professional judgement, and have the following elements : 1) hazard identification, 2) dose-response assessment, 3) exposure assessment, and 4) risk characterisation. **Figure IV-3-1** shows the connection and flow of each of these elements. Hazard identification is a qualification

step which ascertains qualitatively whether there is harm to health and the kind of harm. This is followed by the quantification steps of dose-response and exposure assessment. Finally, the risk characterisation process compares the results of quantification assessments. Environmental monitoring of chemicals should consider the processes of "hazard identification" and "exposure assessment" when research plans are being made. For example, the selection and prioritisation of target compounds : that is, that making a priority list requires information about toxicity, production volumes, and use etc., and when the research site, time, and sampling frequency have been decided, exposure prediction will use environmental behaviour model.

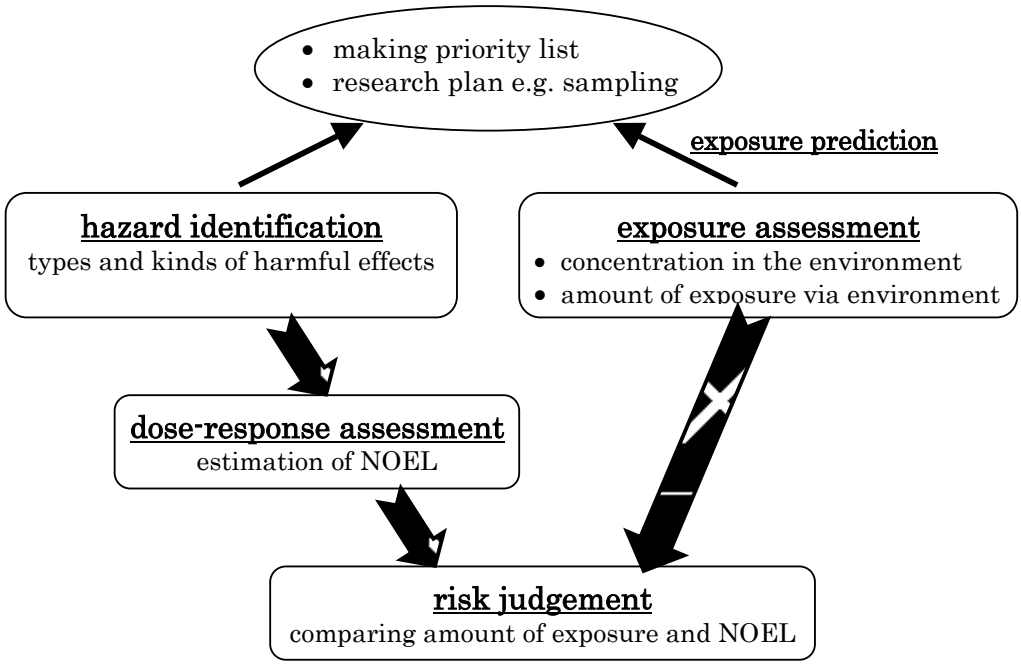


Figure IV-3-1 Factors and the flow of risk assessment

IV.3.1.1 Hazard identification

This step is used to qualitatively identify chemical hazards, in other words to clarify all of the main potential hazards which can happen with human activities. If human epidemiological information can be utilised, hazard identification becomes more efficient. However, one must not stray too far from analysing cause and effect relationships, or mistake or mix up such effects with other causes. But, unfortunately, it is a fact that for most chemicals such hazard information is not known. Therefore, we must predict health hazards based on experimental results using experimental animals.

To predict health hazards, first of all outline the potential hazards using data collected from

fact sheets and data bases. Then detail the hazards by checking the toxicity of each chemical or process by using original or review articles. Review documents published by international organisation or national organisation, groups are useful. For example, RTECS (Registry of Toxic Effects of Chemical Substances) contains acute toxicity, mutagenicity data etc. edited and published by US NIOSH (National Institute for Occupational Safety and Health), IARC Monographs on Carcinogenic Risks to Humans by IARC (International Agency for Research on Cancer), WHO (World Health Organisation), and ICPS (International Programme on Chemical Safety) which at present has published review documents for 170 compounds as EHC (Environmental Health Criteria). Also, the US Department of Health and Human Services has been making review documents (Toxicological Profiles) of 20 or so compounds every year. Analyse and organise obtained information using the following criteria.

A. Human exposure data

Human exposure data is obtained from epidemiological studies of accidental exposure in the workplace or exposure experiments using volunteers. Data obtained from exposure experiments using volunteers can provide quantitative information about dose-response relationships from which TDI (Tolerable Daily Intake) or ADI (Acceptable Daily Intake) criteria can be created, and if there is enough proof, a low safety coefficient. Occupational and accidental exposure can provide useful data to confirm the relationship between apparent harmful effects and dose if it is possible to specify causative agents and estimate the amount of exposure.

B. Toxicity test data using animals

Toxicity tests using animals are used to predict relationships between health effects and chemical characteristics, chemical form, reaction and exposure routes. When information for chemicals of similar structure is available, it is possible to investigate the possibility of toxicity based on the chemical's structure alone.

The purity (or impurity) of the compound used in the animal experiments must be known before the true cause of apparent harmful effects can be elucidated. In addition, the stability and reactivity of the compound itself and its stability in food or water is essential to guarantee the amount of exposure to animals. It is desirable to use animal species of known lineage and which are commonly used in toxicity tests, using sufficient numbers of animals per treatment group to provide statistically significant results. Ideally, use equal numbers of both sexes, rather than either just males or just female. Ensure that both exposure and observation time are appropriate for the experiments. Ideally, dose and general toxicity or toxicity effect on target organs is the

result of a linear dose-response relationship, and NOAEL (No Observed Adverse Effect Level) can be confirmed.

Toxicity testing must consider influences on drug metabolism, the immune system, and internal secretion functions. A knowledge of the target compound's pharmcodynamics, that is, absorption, distribution, accumulation, metabolism, secretion, and also species and sex differences is very useful. Information on pharmacokinetics helps improvement the accuracy of quantitative assessments. Mutagenicity tests, mutation induction tests, chromosome aberration induction test, and micro nucleus test have become common toxicity screening methods for the prediction of carcinogenesis. Results of long term carcinogenicity tests provide the most direct information for confirmation of harmfulness. When the rate of carcinogenicity in the test group administered the chemical is higher than the control group, and the time taken for cancer to develop is short, this is judged a positive carcinogenesis. And when animals of both sex or multiple organs develop cancer, or many kinds of tumour appear, or cancer develops within different species, carcinogenicity is regarded as being confirmed.

The identification or risk assessment of toxicity hazards follows several steps, first collecting information, then evaluating the reliability of the information, and then confirming the data. Generally, the procedures followed are to confirm statistically or biologically the difference between control groups and treated groups, and the correlation between dose and apparent hazard based on the results of many different toxicity tests, such as repeated treatment toxicity tests, teratogenesis / reproduction test, carcinogenesis tests, hereditary toxicity test, etc., all the while checking the effect of target organs and other harmful effects from changes in each test procedure. Then the relationship between response severity and exposure is quantified by scientific methods. Following quantification, a dose-response assessment applies animal data to the human case. The dose-response assessment is often undertaken at the same time as an exposure assessment because they are related to each other.

IV.3.1.2 Quantitative risk assessment

Risk, "P", is a function of the amount of exposure, "D", that is,

$$P = f(D).$$

A dose-response assessment determines either the function "f", the exposure assessment, D, and/or the risk assessment, P. The term quantitative risk assessment is conventionally used to cover all of dose-response assessment, exposure assessment, and risk assessment, but it would be generally better to use the three terms to show the three steps clearly.

A. Dose-response assessment

Dose-response assessment is the second step in conducting chemical risk assessment. Dose-response assessment is used to determine the LOAEL etc. by considering the correlation between chemical intake, the appearance of toxicity and the mechanism of appearance. This step is used to calculate the amount of chemical which does not cause an effect on health when a human takes or is exposed to chemicals. Generally, low dose or environmental exposure dose-response relationships are estimated by extrapolating in the direction of low concentrations data obtained from dose-response relationships produced by high doses in animal experiments or observed under relatively high exposure conditions in epidemiological research. As mentioned later, several extrapolation methods are available, but it is important to choose the most appropriate one.

Dose and amount of exposure are often treated as having almost the same meaning. Sometimes, the terms are synonymous with the amount of toxic agent administered in animal experiments, and sometimes the amount of toxic agent taken into the body calculated from the concentration of the chemical in environmental samples or food. However, the real concentration of chemical at the target organ(s) in the body generally decides the kinds and size of health effects. If the real concentration in the target compartment or organ in the body is understood as the dose (D), the accuracy of the dose-response assessment increases significantly, and a plot of the logarithm of dose against response more often than not shows a linear relationship.

Real concentrations in the target compartment or organ in the body are not only influenced by intake, but also by toxicodynamic conditions, such as chemical absorption rate, distribution, antidotal action, excretion, metabolism activation, connection rate with target, and the fixation rate etc. Understanding the details of these factors is the key to conducting accurate dose-response assessments, and then getting risk assessments right.

Non-linearity of exposure (intake) and response proved by non-linearity of antidotal action or activation becomes influential evidence for the judgement of threshold values. There are two categories of threshold toxicity evaluations which are conducted because of the appearance of toxicity mechanisms assuming that all appearances of toxicity are applicable to humans, except for specialised toxicities to experimental animals.

a) Evaluations in the case where threshold values are assumed to exist

In this first case, threshold values are assumed to exist. In other words, the dose-response curve is not linear. There is a minimum dose that must be absorbed before toxic effects are caused. This minimum dose is the threshold value. Included in this category is carcinogenesis not linked to general toxicity, teratogenesis / reproductive toxicity, and hereditary toxicity. In particular, carcinogenesis by means of chemicals causing cancer indirectly, or secondarily through hormone or

activated oxygen radicals are included in this category. Since it is considered that toxicity does not appear below a certain dose, the threshold levels are usually indicated by the NOAEL. If the NOAEL cannot be determined by toxicity tests or epidemiological research, i.e. only a LOAEL can be determined, the appearance of minute amounts of toxicity can be estimated by applying a safety coefficient (safety factor) (usually 10) to the NOAEL. However, if toxic effects are apparent even at 1/10 of the dose, the LOAEL cannot be used for evaluation. The Benchmark Dose Method (BDM) can be applied to calculations of the NOAEL of general toxicity and teratogenesis / reproduction toxicity. The BDM fits ideal curves to the results of experiments. This allows the determination to 95% confidence limits of a limit on the dose that causes the appearance of 5 to 10 % toxicity within the range of experimental doses. The 5 % toxicity appearance rate for teratogenesis / reproductive toxicity, or 10 % for general toxicity are empirically equal to the NOAEL. The BMD is also being applied for carcinogenesis evaluation.

Values obtained from the NOAEL, LOAEL or BDM can be used to determine the ADI or TDI for humans by dividing such values by a SF (safety factor) and UF (uncertainty factor). Both SF and UF are factors which apply to intake amounts which are assumed to cause no harmful effects. It is therefore OK to treat the ADI or TDI as having the same meaning. In addition, the ADI and TDI can be described as being "the level of harmful compound which it is assumed will cause no harmful effects on human health even if a human being were to take it everyday for its whole life", and are expressed as the intake amount per kg of human body weight per day.

Generally, the terms TF and TDI are used for environmental chemicals to which people are not exposed intentionally, and the terms SF and ADI are used for food additives which are intentionally added to food. The US Environmental Protection Agency (US EPA) uses a Reference Dose (RfD) for ADI, and Reference Exposure Concentration (RfC) to calculate allowed amounts per day.

The terms UF and TDI are used as factors to correlate general human epidemiological research and animal experiments containing the following uncertain factors:

(1) interspecies differences - apply conversion factors from animal to human
(2) interspecies or individual differences - differences between human
(3) different kinds of toxicity information with different reliability used to estimate human effects, such as experimental exposure period and design, the existence of supportive or nonsupportive data etc.

Point (2) is called the "distribution uncertainty." Points (1) and (3) are called the "true uncertainty". The US EPA sometimes calls point (3) the modifying factor (MF). Generally, the MF is 10 for interspecies differences assuming humans are more sensitive than experimental animals, 10 for interspecies differences taking into account the difference in groups of humans. The default value is the product of these two points i.e. UF = 100. The UF may be subdivided

when a knowledge of toxicokinetics or dynamics reflecting interspecies and individual differences in the experimental data is known. In the following cases, a maximum of 10 is added.

- when toxicity observed near the NOAEL is serious, such as liver cell death
- when the quality of the toxicity test is not good. For example, animal numbers are small, experimental conditions are insufficient, reliability of data is low, etc.
- when only an NOAEL is obtained. However, additional UF are not necessary if the BMD can be used.
- short term repeat administration toxicity tests. In general, a factor of 10 is applied for toxicity test of 3 months to 2 years, or, for example, when data from important toxicity tests such as teratogenesis / reproducibility toxicity test are not available.

For evaluation of carcinogenesis not linked to hereditary toxicity, a UF10 is added when toxicity is associated with tumours, but no addition of a UF is required for a non-tumour associated disease. On the other hand, for NOAELs obtained from occupational exposures equal to a 90 day inhalation exposure experiment, the TDI is usually calculated as UF3 - 10. This is because highly sensitive groups such as old people, children, and sick people are not included in the group of workers, and length and concentration of exposure are accurately understood.

Thus, the UF is determined by considering the kinds of toxicity and experimental design, and the TDI is determined by dividing the NOAEL, LOAEL and BMD by the UF value. Recently, efforts have been made to make the UF as a small value as possible, i.e. to make the TDI more reliable by considering information such as toxicity appearance mechanisms, physicochemical character and structural activity correlations etc. In general, the UF is not supposed to exceed 10000, and if does exceed this value, it cannot be applied to humans.

b) Evaluation in the case assuming threshold values don't exist

When threshold values are assumed not to exist, for instance, in the evaluation of carcinogenesis linked to hereditary toxicity, a Virtual Safety Dose (VSD) is calculated by applying to low dose using mathematical models. The VSD is the dose which is regarded as probably being substantially safe compared with everyday risks, and the values adopted are usually of the order 10^{-6} - 10^{-5} at present. In Japan, 10^{-5} is the current end point following WHO guidelines. In other words, the life time risk that one out of 100,000 people will suffer cancer because of exposure to a particular risk with a statistical probability of 99% is regarded as being practically safe.

The method used to calculate the VSD is to determine a dose which is equal to 10^{-5} or 10^{-6} risk after inserting the relationship between dose and carcinogenic rate (the results of carcinogenesis test which is conducted by several doses) into a mathematical model. Generally, the VSD is estimated by determining a dose which is equal to a certain risk from the reverse function $D = f^{-1}(P)$

of the dose-response function P = f(D) : where, P is carcinogenic rate, and D is the dose.

There is another way to estimated the VSD's 99%, 95% and lower confidence limits. The Linearised Multi-stage Model mentioned later is most widely used at present for the evaluation of carcinogenesis risk. When this model is used, the VSD is calculated from experimental results obtained computer simulations, determining the biggest value, q*, of the slope at the low dose range, and then dividing a certain risk level, for example 10^{-5}, by q*.

There are also mathematical models such as Probit and Logit which are based on probability distribution, One-Hit, Multi-Hit, Weibull, Multi-Stage Model, and Linearised Multi-Stage Model which are based on carcinogenicity mechanisms.

- Probit Model : In this model, the logarithm of acceptable intake, or of toxic response, of each chemical follows normal distribution whose parameters are the average, μ, and the standard deviation, σ. In this model, the dose-response curves becomes sigmoid when D becomes close to 0 and P becomes almost 0, and when D becomes larger and P becomes close to 1.

$$P = \varphi[(\log D - \mu)/\sigma] = \varphi(\alpha + \beta \log D)$$

φ : standard normal distribution function (accumulation something distribution function),

$\alpha = -\mu/\sigma$, $\beta = 1/\sigma$

- Logit Model. Although, like the Probit Model, this model gives sigmoid dose-response curve when D becomes close to 0, P becomes 0 more slowly than Probit Model.

$$P = 1/[1 + \exp\{-(\alpha + \beta \log D)\}]$$

- One-Hit Model. This model assumes that one hit by a toxic agent during a certain time period causes cell defects which cause tumours. At low doses, the slope shows linearity of λ[I - f(D)].

$$P = 1 - \exp(\lambda D)$$

λD : expectation of hit numbers

- Multi-Hit Model. This is the generalised version of the One-Hit Model, and assumes that cells becomes cancerous after being hit more than n times during a certain period. At low doses, the formula can resemble $P = \lambda D^n$, and when n = 1, this model gives linear dose-response curve, n > 1, curved downwards, and n < 1, curved upwards.

$$P = 1 - \sum_{k=0}^{n-1}\{\exp(-\lambda D)(\lambda D)^k / k!\} = \int [\prod_{0}^{D}(n)]^{-1}\lambda^n t^{n-1}\exp(-\lambda t)dt$$

- Weibull Model. Another generalised version of the One-hit Model. At low doses, when n = 1, this model gives a linear dose-response curve, n > 1, curved downwards, and n < 1, curved upwards.

$$P = 1 - \exp(-\beta D^n)$$

- Multi-Stage Model. Generalised version of Multi-Hit Model. In this model, the effects of target compounds at several stages are associated with other stimulations occurring at each stage.

$$P = 1 - \exp[-\prod_{i=1}^{\infty}(\alpha_i + \beta_i D)]$$

$$(r_i \geq 0)$$

- Linearised Multi-Stage Model. This model was developed from the Multi-Stage Model and shows linearity in the low dose range. When slope of the line is q*[(mg/kg/day)$^{-1}$], it becomes the following formula.

$$P = q^*D$$

where q* is the maximum value of the slope at the low dose range, and is equals to the 95% upper confidence limits of this model. This value is widely used as an index of the intensity of carcinogenesis of carcinogens.

The risk of carcinogenicity at low doses is obtained from animal experiments, the data from which must be applied to human doses, and the many interspecies differences considered. In general, comparisons based on body weight or body surface area are common, but it is not clear which is most appropriate. The US EPA thought it appropriate to consider body surface area (proportional to body weight_raised to 2/3rd power) when the doses used in animal experiments were applied to humans. This decision was based on the experimental results that the dose at which toxicity appeared becomes equal in both animals and human when the dose is not indicated 'per unit of body weight' but by 'per unit of body surface area. However, later the US EPA suggested that body weight raised to 3/4th power was more appropriate than body surface area ratio.

For the evaluation of carcinogenic risk, when the dose causing tumour appearance is not clear, a UF is sometimes used. For example, the maximum dose which doesn't cause cancer is divided by 5,000, adding a UF50 for carcinogenesis linked to hereditary toxicity. Also , there is another way to compare amounts of exposure which cause 5% tumour appearance rates in animal experiments or epidemiological research and amount of human exposure.

B. Exposure assessment

Chemical exposure is defined as chemical contact with the internal and/or external surfaces of a human or other organism. Exposure assessment aims to estimate the total amount of exposure by clarifying the structures and sizes of exposed groups, and by considering exposure routes, the frequency, and the periods during which chemicals in the media directly expose the environment and human beings. By comparing the levels obtained from dose-response assessments, the safety (or danger) of the present state of pollution is assessed. Knowledge about the reality of pollution and exposure obtained from exposure assessment is also useful for strategies such as risk management with end points of the efficient reduction of chemical risks. In this section the processes by which data obtained from environmental monitoring are introduced into exposure assessment are described.

a) Chance and routes of exposure

Chemicals discharged into the environment (**Figure IV-3-2**) are distributed in the atmosphere, water, soil and the biosphere by way of transfer and diffusion. In addition, there are some compounds which are bioconcentrated through the food chain in the biosphere. Human beings take in chemicals from the various environmental media such as food and drinking water, via the inhalation, oral, and dermal routes. In order to determine the amount of human exposure, calculate the amount exposure from chemical concentrations in the exposing media and the intake via each route, and finally add up these values to obtain the total amount of exposure from all routes. Finally, conduct an assessment of amount the exposure by considering the continuance of exposure, i.e. period, frequency, and timing. Chemicals are generally reported for environmental monitoring purposes as concentrations in environmental media such as the atmosphere, lake water, river water, sea water, underground water, sediment, soil, seaweed, mussels, fish, birds, etc.. One must pay attention to differences between environmental media, and media which expose humans, when such data is combined for exposure assessment.

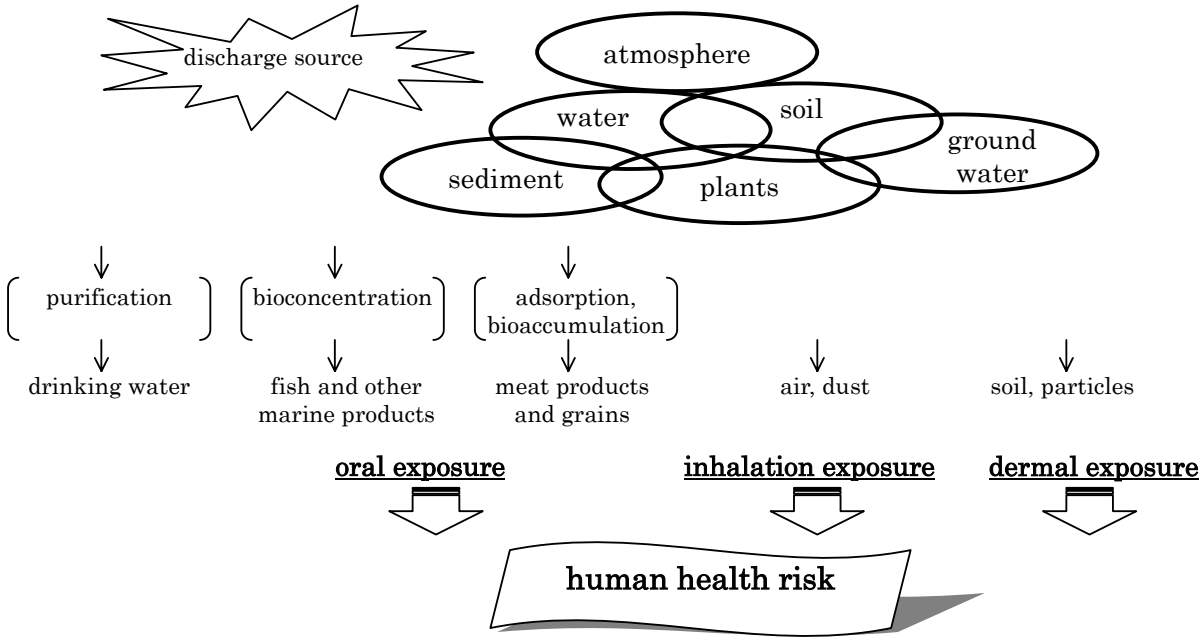


Figure IV-3-2 Exposure route of environmental chemicals to human

(1)Oral exposure

Sources of oral exposure to humans are drinking water, food and dust. The primary source of drinking water in developed nations is tap water, which is supplied after river, lake, and ground water have been purified by such treatments as aggregation, precipitation, filtration, and sterilisation etc. Most raw food raw materials, such as fish, sea products, vegetables and grains, meat, dairy products are treated by washing, processing, and cooking. Environmental monitoring data cannot be used directly for exposure assessment because some chemicals are concentrated, diluted, or decomposed during such treatments. Therefore, analysis of actual exposure media is desirable, since the accuracy of assessment becomes higher, but limitation of time or budget also happens.

Concentrations of chemical compounds in tap water can be estimated with a knowledge of their behaviour in the water purification process. For example, chemicals whose water solubility exceeds 10 μg/mL are difficult to remove by aggregation / precipitation or sand filtration, sulphated compounds are easily oxidised and decomposed, low molecular weight halogenated compounds such as trihalomethane can be produced in treatment processes, etc. For chemicals where such information is not known, it is necessary to analyse actual drinking water. Generally, analytical methods for river and lake water can be applied to drinking water.

Table IV-3-1 Average intake by food category in Japan (g/day/person) 1975～1995.

food category	1975	1980	1985	1990	1993	1994	1995
grain, rice	248.3	225.8	216.1	197.9	195.4	192.4	167.9
Wheat	90.2	91.8	91.3	84.8	86.9	86.4	93.7
Potatoes	60.9	63.4	63.2	65.3	62.5	62.2	68.9
Oil	15.8	16.9	17.7	17.6	17.9	17.6	17.3
Beans	70.0	65.4	66.6	68.5	65.9	66.8	70.0
green vegetables	48.2	51.0	73.9	77.2	81.6	81.8	94.0
other vegetables	198.5	200.4	187.8	173.1	180.6	171.7	196.2
Fruits	193.5	155.2	140.6	124.8	114.9	117.2	133.0
Seaweed	4.9	5.1	5.6	6.1	5.5	5.8	5.3
Sugar	14.6	12.0	11.2	10.6	10.2	10.0	9.9
soft drink	119.7	109.4	113.4	137.4	143.3	147.7	190.2
Sweets	29.0	25.0	22.8	20.3	20.3	19.6	26.8
Fish, sea products	94.0	92.5	90.0	95.3	96.2	97.0	96.9
Meat	64.2	67.9	71.7	71.2	73.7	74.5	82.3
Eggs	41.5	37.7	40.3	42.3	42.7	43.0	42.1
milk, dairy products	103.5	115.2	116.7	130.1	130.8	132.4	144.4

Human beings eat about 90 different kinds of foods, and for many chemicals these are the main exposure routes. Foods originate from other living things, and these living things end up on the table after adsorbing or concentrating chemicals discharged into the environment. In order to understand the amount of exposure from food accurately, it is necessary to know the amount of chemical in all foods prepared for eating. There are only a limited number of foods or food groups which have high concentrations of chemicals (**Table IV-3-1**). Therefore, after choosing the food or food group which has high concentrations of chemicals, the origin and amount of exposure through food can be determined from chemical concentrations in the food and the daily intake (**Table IV-3-1**). Although the range of environmental monitoring results becomes ever wider and additional analysis is still needed, this method has the advantage of being able to obtain accurate analytical values relatively easily because sample matrices are often homogeneous.

The amount of chemical exposure from foods in which the level and distribution of contamination is unknown has to be determined by investigations of whole foods. Commonly used methods are market basket method and the indirectly table method. The former is often used because sample collection is easier. Put simply, the method is to purchase everyday foods from every food group of **Table IV-3-1** based on national average food intakes, cook them using ordinary cooking methods, and homogenise. The homogenised food samples are the direct exposure media, and the average amount of chemical exposure can obtained from chemical analytical data. The indirectly method takes a single meal and homogenises it. This homogeneous mixture is then analysed. Both methods can provide data for accurate and convincing exposure assessments.

However, these methods analyse the whole food from which the meal is prepared, and it is often very difficult to deal with materials such as grains, vegetables, fish and sea products, meat, dairy products, etc.

Dust originates from particles or smoke discharged from factories and motor vehicles, soil particles, particles from the sea, chemicals themselves or chemicals attached to the surface of clay particles or salts. Humans inhale these dusts through the mouth and nasal cavity. Dusts in the air are also attached to food or hands, and get into human bodies by such motions as putting hands in the mouth or eating a meal. Dusts entering the nasal cavity can reach lung alveoli through the airways of the throat (the larynx and pharynx), trachea, and bronchus, but most of the dust sticks to airway mucous membranes, and are moved back to mouth as sputum, or reach the digestive organs via the gullet. In general, dusts with a diameter of more than 0.1 - 1.0 μm are assumed to stick to the upper or lower airways, and can be regarded as oral exposure media. Thus, oral exposure by dusts is through every day, unconscious motions, meals and breathing. Of these, exposure through meals is difficult to calculate because of many of indeterminate factors. Therefore, although there is possibility of overestimation, it is most practical to determine the amount of oral exposure through dusts from the Total Suspended Particulates (TSPs) in the atmosphere, chemical concentrations and aspiration rate. The TSP can be replaced by a measure of Suspended Particulate Materials (SPM). SPMs are particles with a diameter less than 10 μm. Exposure through actions such as putting soil in the mouth is also of concern, particularly for small children, therefore data detailing soil chemical concentration are also useful.

(2) Inhalation exposure

Humans may be exposed to chemicals in the atmosphere via inhalation of both gases and particles depending on such physicochemical characters as vapour pressure, adsorption isotherms to organic matters. Gaseous chemicals are assumed to reach lung alveoli via nasal cavity or mouth, but atmospheric particles form all or a part of the SPM. The diameter of the particles is as much a problem for inhalation exposure as it is for oral exposure from dusts, and the SPM which reaches alveoli without sticking to the mucous membranes of the nasal cavity, throat, or airways can be defined as the inhalation exposure media. In general, they are micro particles with a diameter of less than 1.0 - 0.1 μm.

Human behaviour, particularly living and working patterns must be considered when assessing atmospheric exposure. In general, humans spend only 1 - 3 hours outdoors, but more than 10 hours working and relaxing in the indoor environment. Therefore, the amount of inhalation of indoor environmental air is the overwhelming majority. In addition, some chemical concentrations are higher in indoor air than in the general atmosphere, so an understanding of the amount of exposure to indoor air is indispensable for calculating the true amount of exposure

and evaluating health effects. Basically, the amount of exposure is determined by measuring chemical concentrations in indoor air and the atmosphere outdoors and allocating residence times At this time, the contribution of the general environment and the indoor environment to total risk has to be clarified in order to estimate heath effects brought by environmental pollution.

(3) Dermal exposure

Soil is the main source of dermal exposure. In general, the results of soil analyses as part of environmental monitoring can be used as the exposure concentration.

b) Duration of exposure

Risk assessment of environmental pollutants targets health effects which can be caused by intermittent exposure to wide range of people for the whole of their life span, i.e. 24 hours per day for 70 years is one standard. However, when exposure from some daily patterns of activity are significantly different, such as indoor exposure, and cannot be ignored, the amount of exposure for each environment has to be calculated and added together. In addition, the timing of exposure can be an issue, so compounds which give produce effects in a certain age group have to have the amount of exposure in that period estimated separately. Thus, when a certain group has specified exposure and sensitivity, it is necessary to determine distributions depending on region, occupation, age, or sex. The results of exposure assessment should be reported in the same dose units are used for dose-response assessment because judgements on risk are conducted by combining exposure assessments with results of dose-response assessments. Many of them use intake per unit body weight (kg).

IV.3.2 Application of environmental monitoring data to exposure assessment

Data obtained from environmental monitoring would have big change as timewise and spacewise. It is, therefore, a problem which exposure concentration - mode, mean, geometric mean, or median - should be used, and how to treat values which are less than detection limits.

The results of environmental monitoring are used to assess health risks due to continuous and long term exposures at low concentrations. Thus, it is the most appropriate to use values such as the mean or median. However, some evaluations using the mode or confidence limits of the data are also necessary when, for example, a population is partially exposed to high concentrations. In addition, if multiple peaks appear in the histogram of data, the reason has to be investigated and division of the exposed group has to be considered. Furthermore, if there is a difference in

exposure status as a result of the source of an outbreak, regionally or dietary differences etc., or groups of different sensitivity are found, it is necessary to select the most appropriate data or look at reorganising the groups.

There is no problem in abandoning detection limits at the data analysis stage, or if detection limits are within the acceptable risk range, but there is the possibility that exposure at the detection limit cannot be ignored depending on the behaviour of the target chemicals. In this case, one way to evaluate the amount of exposure is to set temporary detection limits at 1/10th concentration. On the other hand, it is always desirable to improve analytical detection limits since both analytical methods and quality control techniques are very important and should sufficiently robust to facilitate representative or full data comparison when the data is the basis of risk evaluation.

When exposure assessment procedures based on environmental monitoring results start, the necessary data is often unavailable and estimates of environmental concentrations are required. Such estimates are, as mentioned in the previous section on behavioural analysis, based on regularity or connection between other appropriate environmental concentration data and physicochemical characteristics such as water solubility, vapour pressure, octanol-water partition coefficient, etc., information on hydrolysis, photodegradation, biodegradation, bioconcentration etc., and environmental conditions such as geography, weather, etc. However, in general estimated values are inferior to experimental data in terms of accuracy, and it is necessary to include this uncertainty.

IV.3.2.1 Quantification of amount of exposure

The amount of exposure is determined by using environmental monitoring data, data from direct human exposure data, or estimated values based on compound behaviour. Chemical concentrations at the contact points in the mouth, nasal cavity and skin is deemed to be the exposure concentration, and the amount of exposure within a certain time E_{total} can be determined by the following formula：

$$E_{total} = \sum_{m=1}^{n} \int_{0}^{t} C_m(t) I_m(t) dt$$

where, E_{total} is the amount of exposure between voluntary time 0 - t, $C_m(t)$ is the chemical concentration in the media , m, is the inhaled, ingested, or absorbed per time t, $L_m(t)$ is the amount of inhalation, ingestion, or contact surface area in the media, m, per time t. Environmental monitoring data can be used directly or indirectly to determine chemical concentration, but in such a case would be the potential concentration which may be absorbed directly if the compound's

adsorption rate (bioavailability) is 100 %. A chemical compound has to be adsorbed through the skin, lungs, or digestive organs in order to produce a toxic effect, and to estimate a compound's inherent adsorption function, information for each exposure route is necessary. In addition, toxicity can be the result of a specific interaction between absorbed chemicals and a biological receptor. If part of the absorbed dose is transferred to each organ, tissue, and bodily fluids, then very little chemical actually reaches the target cell, receptor, or membrane, and little disease develops. If the true amount of exposure or true concentration is estimated by explicitly considering information about chemical movement in the body, highly accurate evaluations becomes possible, but such data is not readily available.

A. Oral exposure

The media responsible for oral exposure, drinking water, food and dust, are considered to produce an amount of exposure determined by the following formula :

$$E_{ingestion}\ (\mu g/\ day) = \alpha \cdot C_{water} \cdot I_{water} + \beta \cdot \sum_{i}^{n} Ci_{diet} \cdot Ii_{diet} + \gamma \cdot C_{dust} \cdot I_{dust}$$

where, $E_{ingestion}$ = oral exposure amount per day (μg/day)

C_{water} = target compound concentration in drinking water (μg/L)

I_{water} = drinking water intake per day (L/day)

Ci_{diet} = target compound concentration in food i (μg/g)

Ii_{diet} = food i intake per day (g/day)

C_{dust} = target compound concentration in dust in the environment (μg/g)

I_{dust} = orally taken dust amount per day (g/day)

α, β, γ = absorption rate in digestive organs of target compounds in drinking water, food, and dust

In general, the amount of drinking water required per person per day is 0.5 L for infants and 1.2 - 1.5 L for adults, and often exceeds 2.0 L depending on a particular occupation or sports regime. The WHO, EPA, and Japan calculate that the amount exposure for an adult from drinking water as being from a standard 2 L of water. In addition, the EPA considers the amount exposure for an child as being from a standard 1 L of drinking water for a child weighing 10 kg. The amount of exposure from food is determined as the sum of the product of the concentration of each target chemical in each food and the amount of intake, after selecting foods which are predicted to have the highest contamination levels and greatest amount of intake. If data on the amount of

exposure through market basket methods are available, then it is OK to multiply the amount of exposure by absorption rate. Although the amount of dust taken orally per day changes a lot, from the average human daily inhaled volume (15 m^3/day, proportional to the2/3 rd power of body weight, the US sets inhaled volumes at 20 m^3/day for a standard 70 kg body weight), it can resemble the chemical concentration in TSP (or SPM), and TSP (or SPM). In addition, intake of dust or soil is estimated to be 0.0001 g/day in the relatively clean, enclosed environment, 0.05 g/day for workers in general industries, 0.48 g/day for workers on construction sites, and 10 g/day when in contact with dusty ground all the time. The intake of dust or soil in the general living environment is estimated to be 0.001 - 0.01 g/day, and by taking into account chemical concentrations in soil, exposure can be predicted. Digestive absorption rates, α, β, and γ, are compound dependent values, and they, even for the same compounds, are different depending on the nature of each medium. However, details are generally unknown. If sufficient scientific knowledge is available, the WHO adopts it, and if nothing is available, treats absorption as being 100 %, and gives an absorption rate of 1.

B. Inhalation exposure

The media responsible for inhalation exposure, air and suspended particulate materials, are considered to produce an amount of exposure determined by the following formula :

$$E_{inhalation}(\mu g/day) = \delta C_{air} I_{air} = (\varepsilon C_{gas} + \xi C_{fine} SPM_{fine} 10^{-3}) I_{air}$$

where, $E_{inhalation}$ = inhalation exposure amount per day (μg/day)

C_{air} = target compound concentration in air (μg/m^3)

C_{gas} = target compound (gas state) concentration in air (convert ppb to μg/m^3)

C_{fine} = target compound concentration in suspended particulate materials (μg/g)

SPM_{fine} = suspended particulate material amount in air (mg/m^3)

I_{air} = breathing amount per day (m^3/day)

δ, ε, ξ = alveoli absorption rate of target compounds

10^{-3} = correction factor (g/mg)

Chemical concentrations in the atmosphere are expressed using the weight-capacity unit, μg/m^3, when chemicals exist in the particulate state, and the capacity-capacity unit, ppb (part per billion), when the chemicals exist in the gaseous state. When risk assessment is conducted together with dose-response assessment, the weight-capacity unit is convenient for the results of exposure assessment, and conversion is done using the following formula :

$$\mu g / m^3 = ppb \frac{g-mol}{22.4L} \frac{MW}{g-mol} \frac{273.16K}{T} \frac{P}{hPa} \frac{10^3 L}{m^3} \frac{10^3 mg}{g}$$

where, ppb = target compound concentration (L/10^9L), MW = weight (g) of 1 g mol of target compound, 22.4 L = occupied by 1 g mol compound at 0 °C, and 1 atm (1,013 hPa), T = temperature (Kelvin), P = atmospheric pressure (hPa).

Under standard conditions, for example, 25 °C, 1 atm, the volume of perfect air 1 g mol becomes 24.45 L, and the above can be simplified to the following formula：

$$\mu g/m^3 = ppb \cdot \frac{MW}{24.45}$$

Generally the amount of inhalation exposure ($E_{inhalation}$) can be determined from the product of chemical concentration in air (C_{air}), the amount inhaled (I_{air}), and the absorption rate (δ). There is only limited analytical data in which chemicals in the air are divided into gaseous and particulate states, and where C_{air} is the total concentration. If such data can be used, the amount of exposure would be sum of both. Then, if particle size is limited to the less than 0.1 - 1.0 μm SPM fraction, and in the formula chemical concentration in suspended particulate materials is expressed as C_{fine}, the amount of suspended particulate material is SPM_{fine}. The amount inhaled per person per day is calculated as 150 m^3/day (standard body weight 50 kg) in Japan, with a range of 10 - 20 m^3 depending on body weight or the amount of exercise taken. This inhalation volume is based on a breathing rate of 20 L/min for 16 hours a day, and 7.5 L/min for 8 hours a day. The alveoli absorption rates, δ, ε, and ξ depend on compounds and the matrix, but if a value is not available, 100 % is used as the default value supplying a worst case scenario. The correction factor 10^{-3} is a unit adjustment value.

C. Dermal exposure

The amount of exposure through skin contact with ground surface deposits such as soil particles can be calculated by the following formula：

$$E_{contact}\ (\mu g / day) = \eta C_{deposit} SA \cdot SAF \cdot 10^{-3}$$

where, $E_{contact}$ = dermal exposure amount per day (μg/day)

$C_{deposit}$ = target compound concentration in ground surface deposit (μg/g)

SA = exposed skin area per day (cm^2/day)

SAF = skin adhesion factor (mg/cm^2)

η = skin absorption rate

10^{-3} = correction factor (g/mg)

The amount of dermal exposure ($E_{contact}$) is calculated from the product of chemical concentration in the ground surface deposit particles ($C_{deposit}$), attaching surface area per day (SA), skin adhesive factor of particles (mg/cm^2), and adsorption rate through the skin (η). Chemical concentration from environmental monitoring is used. The EPA uses a standard human body surface area, i.e. skin area, of 18,000 cm^2 for a 70 kg person, and because it is proportional to 2/3rd power of body weight, it becomes 14,000 cm^2 for body weight of 50 kg, or 16,000 cm^2 for a body weight of 60 kg. It is not known how much skin is exposed during everyday life, but it is assumed to be 1/3 of the total surface area for exposure assessment. Therefore, the area of skin to which ground surface deposits, such as soil can be attached daily is calculated as 4,8000 cm^2 for a 50 kg body weight, or 5,400 cm^2 for a 60 kg person, and 6,000 cm^2 for a 70 kg person. The skin adhesive factor is the amount of particles that attach per unit area of skin. If no data available, a value of 1 mg/cm^2 can be used. If no data is available on chemical adsorption rates through the skin, the amount of exposure can be calculated by using values of 1 % for organic compounds, and 0.1 % for inorganic compounds. A correction factor of 10^{-3} is a unit adjustment value.

D. Total exposure amount

The amount of total exposure is the sum of oral, inhalation, and dermal exposures.

$$E_{total}\ (\mu g / day) = E_{ingestion} + E_{inhalation} + E_{contact}$$

The value obtained is the amount of exposure per day, and is converted into a value for daily exposure per body weight in order to compare with TDI etc. for risk characterisation. In Japan,

$$E(\ \mu g / kg / day) = \frac{E_{total}\ (\mu g / day)}{BW(kg)}$$

where the standard body weight is 50 kg.

IV.3.2.2 Risk characterisation

Risk characterisation is the last step in the risk assessment process, i.e. the process of assessing the results of hazard identification, dose-response assessment, and exposure assessment, and estimating the extent of risk to humans. Risk manager communicate based on this information. Risk assessment takes place using the whole range scientific knowledge at the time of assessment,

but it is also clear that it is made up of many uncertain factors. Therefore, the results obtained are not absolute and don't guarantee safety. Assumptions in risk assessment include the following :

- Toxic effects in animals appear in human as well.
- Absorption, distribution, metabolism, and excretion are the same in humans as in animal models.
- Carcinogenesis linked to hereditary toxicity does not have a threshold value, but the appearance other toxic effects has threshold values.
- The difference in sensitivity between experimental animals and human is about 10 times.

Usually such data cannot actually be obtained, so the concepts have to be assumed. Therefore, it is necessary to obtain higher reliability by using data for similar chemical compounds where possible, and the premises upon which evaluation is based have to be written into the risk characterisation process. On the other hand, usually risk assessment assumes life time continuous exposure, so risk managers have to understand this point and take social needs into account during regulation preparation, risk diminishment policy etc.

A. Evaluation in the case that threshold values exist

For evaluations where threshold values exist, human risk is judged by comparing the determined TDI and actual exposure. Generally, if daily intake is less than the TDI, it is assumed that there will be no health hazard even if a human takes that dose for life. The International Programme on Chemical Safety (ICPS) distributes TDI for each exposure route based either on actually determined or estimated rates of exposure from air, food, and water, determines concentrations in the various media, and uses such concentrations as Guidance Values for assessment. In addition, tap water quality guidelines of the WHO makes a general chemical contribution from drinking water of 10 %.

Because TDI has uncertainty factors, assessment is sometimes done using a range of values of 1/10 to 10 times. This method is useful when deciding the order of priority of risk characterisation or risk diminishment treatment, or to try to adjust international values. However, when TDI is regarded as a range, it becomes difficult to interpret and obtain consensus recognition for the application.

Another method of evaluation in cases where threshold values exist is the Margin of Safety (MOS) or Margin of Exposure (MOE). The MOS has been used for safety evaluation of medicines, and it is determined by dividing the lowest toxic dose by the effective dose. A chemical is judged safer when the larger the value obtained. When this concept is applied to environmental

chemicals, the NOAEL obtained from toxicity tests or epidemiologic research is divided by the amount of human exposure. The EC uses MOS, the USA uses MOE, and the use of the methods are not the same. The MOS used by the EC is applied to evaluation of high production chemicals by the Organisation for Economic Cooperation and Development (OECD). That is, the NOAEL is indispensable for MOS calculation, and addition of an toxicity test itself is required when the kinds and quality of toxicity tests are not sufficient. Also, when MOS becomes, for example, less than 100, the reliability of exposure data has to be re-evaluated and if necessary re-investigation of the real exposure situation has to be conducted. If it can be confirmed that MOS is small, eventually, risk diminishment treatment is conducted. On the other hand, the US EPA suggests use of MOE for evaluation of carcinogens not linked to hereditary toxicity. The MOE is determined by dividing the amount of exposure by the LED (confidence limits lower value of dose which increase abnormality at 1 % or 10 %) obtained by Benchmark Dose method or the median. When MOE is evaluated, use the way of thinking of UF.

B. Evaluation in the case that threshold values don't exist

For evaluation of carcinogens which cause toxicity but without threshold values, i.e. have hereditary toxicity, the amount of exposure risk is usually evaluated by determining intake and VSD at the risk level of 10^{-5} - 10^{-6}, then comparing the values obtained with a control. In principle, VSD is determined by selecting an appropriate mathematical model based on the mechanism by which cancer develops, and inserting a low dose into the experiment of less than 1/10,000. However, since vital investigation of the mechanisms of cancer development have not been undertaken, VSD has extremely large uncertainty. Incidentally, values of VSD may be separated by up to two orders of magnitude depending on mathematical models. Because of these large uncertainties, the risk of carcinogenesis does not consider individual differences in sensitivity and the rate of contribution of each exposure route. VSD has to be understood as a value which indicates the intensity of carcinogenesis.

IV.3.3 An example of risk assessment - Dioxins

IV.3.3.1 Hazard identification

A. Outline of dioxins

Dioxin is the general name given to the polychlorodibenzo-p-dioxin (PCDD) family of

compounds, and often the context in which this descriptor is used includes the polychlorodibenzofurans (PCDFs). Dioxins are unintentionally produced by-products of certain chemical synthetic processes, and incineration processes. There are 75 PCDD isomers (c0ngeners) and PCDF 135 isomers (**Table IV-3-2**) The aqueous solubility, vapour pressure, degradation character (photodegradation, photooxidation, hydrolysis) of dioxins decreases as the number of substituted chlorine atoms on the rings increases. Dioxins with more than four substituted chlorines are bioconcentrated, global pollutants because of their high lipid solubility (lipophilicity) and stability.

Table IV-3-2 Isomer numbers of PCDD/Fs

Substituted chlorine numbers	PCDDs			PCDFs		
	molecular formula	M.W.	number of isomers	molecular formula	M.W.	number of isomers
1 (Mono)	$C_{12}H_7O_2Cl$	218.64	2	$C_{12}H_7OCl$	202.64	4
2 (Di)	$C_{12}H_6O_2Cl_2$	253.08	10	$C_{12}H_6OCl_2$	237.08	16
3 (Tri)	$C_{12}H_5O_2Cl_3$	287.53	14	$C_{12}H_5OCl_3$	271.53	28
4 (Tetra)	$C_{12}H_4O_2Cl_4$	321.97	22	$C_{12}H_4OCl_4$	305.97	38
5 (Penta)	$C_{12}H_3O_2Cl_5$	356.42	14	$C_{12}H_3OCl_5$	340.42	28
6 (Hexa)	$C_{12}H_2O_2Cl_6$	390.86	10	$C_{12}H_2OCl_6$	374.87	16
7 (Hepta)	$C_{12}HO_2Cl_7$	425.31	2	$C_{12}HOCl_7$	425.31	4
8 (Octa)	$C_{12}O_2Cl_8$	459.75	1	$C_{12}OCl_8$	443.75	1

The main absorption routes of dioxins are through the digestive organs, the skin, and the lungs. Regardless of the absorption route, in general the greater the chlorine substitution of a dioxin isomer, the lower the rate of absorption rate. Absorbed dioxins reach the tissues by means of the blood stream, and are accumulated in the liver and fat stores.

Dioxins stimulate the production of metabolic enzymes, e.g. the cytochrome P450, produced by the liver etc. such as CYP1A1 or CYP1A2 etc. through the aromatic hydrocarbon (Ah) receptor. The enzyme CYP1A1 is especially efficient at combining with dioxins. Cytochrome P450 is not only involved in chemical metabolism, but is also implicated in metabolic processes that promote carcinogenicity and the development of true cancer sources. The mechanism of action of these enzymes and dioxins is related to mechanisms of dioxins accumulation in the liver and the appearance of toxicity. Factors which determine movement of 2,3,7,8-TCDD in the body are solubility and diffusion velocity to fatty tissues, combination with CYP1A2 in the liver, excretion, and metabolism.

The results of animal experiments have shown that dioxins have manifold toxicities, such as acute toxicity, chronic toxicity, carcinogenesis, reproduction toxicity, teratogenesis, immune system

toxicity etc. All these toxicities are not restricted to a single species, but are different depending on animal species, lineage, age, sex etc.

According to current information, the promotion of cancer by dioxins is generally accepted, but since dioxins don't cause breaks in DNA strands directly, and various mutagenesis and hereditary toxicity test are negative, the cancer developing mechanism of dioxins is judged to have threshold values.

The effects of dioxin on humans include serious chloracne caused by excessive occupational or accidental exposure, liver damage, nervous symptoms, effects on respiratory organs etc. Long-term (continuous) health effects include chloracne, but there is no proof that dioxins cause reproductive toxicity in humans. However, the IARC reclassified 2,3,7.8-TeCDD up from a Group 2B carcinogen (possible cancer causing agent in humans) to a Group 1 (human carcinogen) at the working committee in February 1997. Other dioxins are classified as Group 3 (not categorised at present).

B. Examination of NOAEL by animal experiments

Table IV-3-3 summarises the results of a detailed search of all animal experiments conducted using dioxins, which observed effects at the lowest dose and were able to evaluate the NOAEL or LOAEL. Teratogenesis and immune toxicity can be observed at higher dose levels.

Table IV-3-3 Summary of results of animal experiments about 2,3,7,8-TeCDD

Toxicity	animal species	observed health effects	NOAEL (pg/kgBW/day)
Chronic toxicity	Swiss strand mouse	amyloidosis, dermatitis	1,000 (LOAEL)
Carcinogenesis	SD mouse	liver hyperplasia nodule (benign tumour)	1,000
		liver cancer	10,000
Reproductive toxicity	SD rat	fall of conception rate, low body weigh to new born	1,000
	red haired monkey	endometriosis	126 (100-180) (LOAEL)

Experiments using three different kind of animals give the same NOAEL or LOAEL. Within these, oral dose tests using the Swiss strand mouse for one year caused male amyloidosis (starch like degenerative high molecular compounds attach to tissues such as kidney) and dermatitis. The lowest dose 7 ng/kgBW/week (equivalent to 1 ng/kgBW/day) is regarded as the LOAEL.

Long term tests using the SD rat (mixed feed, 105 weeks) gave almost the same results, "liver cancer development at 70-100ng/kgBW/day", as the long term administration tests (forced administration, 104 weeks) using the OM rat by the NTP (US National Toxicity Evaluation Plan).

The results were significant, giving clear dose-response relationships between tested and control groups, and were highly reliable.

The results of a three generation reproduction test using the SD rat were an extreme fall in rates of conception at 100 ng/kgBW/day, inter-uterine death at 10 ng/kgBW/day, and growth defects in the new born, etc. The effects appear at 10 ng/kgBW/day for the F1 and F2 generations. Putting these together, the NOAEL is given as 1 ng/kgBW/day.

Reproductive toxicity tests using the red haired monkey suggested that the control group, and test groups given 5 ng/kg feed (equivalent to 126 pg/kgBW/day dose) and 25 ng/kg feed, show endometriosis at 22, 71, and 86 %, respectively. Classifying by the degree of seriousness, middle level endometriosis didn't appear in the control group, but 43 % and 71 % appeared in the groups given doses of 5 ng/kg and 15 ng/kg - a significantly high result. From these results, 126 (100 - 180) pg/kgBW/day is regarded as the LOAEL, with effects appearing at the lowest level. Other tests using series of red haired monkey support these results. When females were raised with a diet containing 50 ng/kg 2,3,7,8-TeCDD for seven months (equivalent to a dose of 1.26 ng/kgBW/day), two out of eight didn't conceive, four out of six pregnant monkeys had miscarriages, one out of two newborns was immature, and only one was normal.

Another report suggests that there is no significant difference in fecundity between control groups and groups fed a diet containing 5 ng/kg 2,3,7,8-TeCDD (equivalent to 126 pg/kgBW/day dose) for seven months, but after raising the dietary concentrations to 25 ng/kg (equivalent to 630 pg/kgBW/day dose) three out of eight animals didn't conceive, three out of five pregnant animals had miscarriages, one died during pregnancy, and there was only one normal birth.

These results prove that endometriosis observed in red haired monkeys fed a diet containing 5 ng/kg 2,3,7,8-TeCDD is one of the causes of miscarriage and sterility. Understanding the appearance of endometriosis is important and cannot be ignored because there is a dose-response relationship apparent in the results of these experiments, and this test is conducted using primates which have the closest half life and body burden of dioxins to that of humans.

However, there has been no confirmatory evidence from supplementary experiments, and the rate of appearance of endometriosis in the control groups was also fairly high, so there are a few problems in using this information directly in human health effect evaluations.

C. Examination of results of epidemiological research

The appearance of chloracne etc. is taken as a sign of human health effects of dioxins. In addition, a range of epidemiological research has been undertaken on occupationally exposed workers, accidental sufferers, and veterans exposed to Agent Orange in the Vietnam War.

There is one record of human poisoning by dibenzofurans - that of Yusho disease in Japan and

Taiwan. Taiwanese data suggests Yusho disease caused a delay in muscle growth, bone structure and sexual development, a drop in IQ, and effects on the immune system of newborn babies who were exposed through breast milk or prior to birth through the placenta, but case numbers are small and a complete evaluation has not yet been determined.

D. Conversion to toxicity equivalent values

Much of the available toxicity information on dioxins is mainly restricted to 2,3,7,8-TCDD, and that for other dioxins is rather limited. The toxicity of dioxins is strongly influenced by the number and position of the chlorine substituents attached to the molecules' ring structures, and the ability of molecules to combine with the receptors. To assess receptor binding potentials and hence toxicity, the toxicity, or potency, of 2,3,7,8-TeCDD and other dioxins (total 17 isomers of PCDD and PCDF which have substituted chlorines at positions 2, 3, 7, and 8) is expressed as a fraction of the toxicity of 2,3,7,8-TCDD. Expressions of TEQ (Toxic Equivalent) in this section indicate values which have been converted to potencies.

IV.3.4 Dose-response assessment

In Japan, health risk assessment index values are established as the TDIs which are desirable and acceptable limits which maintain human health.

IV.3.4.1 Establishment of TDI

Because there is no appropriate human epidemiology data at present, results of cancer development test by long term administration to SD rat were judged as the most reliable, and were adopted for establishment of the TDI. This test looked for significant increases in liver cell hyperplasia nodule and liver cell cancer, and cancroid of hard palate, nasal and lung membranes, after giving a mixed feed to SD rat for 105 weeks. The NOAEL of liver cell hyperplasia nodule and cancer are 1,000 pg/kgBW/day and 10,000 pg/kgBW/day, respectively.

Table IV-3-4 Establishment ground of TDI values (pg/kgBW/day) for dioxins

	liver hyperplasia nodule	liver cancer
NOAEL (pg/kgBW/day)	1,000	10,000
Uncertainty factor	100	1,000
Details : interspecies difference between rat and human	10	10
Individual difference within human	10	10
Significance of effects	1	10
TDI : (NOAEL/uncertainty factor)	10	10

Judging that there is threshold value for dioxins' carcinogenesis, 10 pg/kgBW/day becomes the TDI calculated by dividing the NOAEL by an uncertainty factor based on the results summarised in **Table IV-3-4**. The reason for the significance difference in effects between liver hyperplasia nodules and liver cancer is that liver hyperplasia nodules are benign and reflects the seriousness of liver cancer.

IV.3.4.2 Establishment of health risk assessment index value

The aim of health risk assessment index values are to prevent health effects by conducting environmental pollution reduction policies and to evaluate the amount of human exposure at desirable, maintainable levels. Therefore, attention is paid not only to carcinogenesis but also to the appearance of related effects, and health defects, to establish a health risk assessment index value.

From this point of view, the results of studies of endometriosis in the red haired monkey also have to be considered when establishing a health risk assessment index value because endometriosis data in the red haired monkey shows a dose-response relationship, and although the mechanism of the appearance of endometriosis is not clear, and the relationship between hormones and immune actions are assumed, the Ah receptor is linked to the appearance of toxicity.

At present, no country has directly established index values for dioxin intake using data obtained from red haired monkey experiments, but the Netherlands suggested a new index value of 1 pg/kgBW/day based on those results.(**Table IV-3-5**)

Table IV-3-5 Dioxins TDI values suggested by Netherlands (pg/kgBW/day)

	endometriosis
LOAEL (pg/kgBW/day)	100
uncertainty factor	100
details : interspecies difference between rat and human	2
individual difference within human	5
significance of effects	10
TDI : (NOAEL/uncertainty factor)	1

In Japan, because there have been no supplementary experiments to back up the data obtained form red haired monkey endometriosis tests, and the symptoms appeared in the control group, health risk assessment index values are not calculated directly from such endometriosis data. However, knowing that this is an important issue and important knowledge, the value of 5 pg/kgBW/day or a double uncertainty factor added to the TDI values (10 pg/kgBW/day) became the health risk assessment index value.

At this point there is no clear scientific data available to make a judgement on whether it is necessary to establish health risk index values lower than 5 pg/kg/day. However, there are some reports of lower dose experiments with results that suggest some health risks such as change of lymphocyte, increase in rates of death caused by influenza infection, decreased learning ability, decrease in reproductive organ weight and sperm production. Therefore, it is important to make an effort to accumulate scientific knowledge about dioxins, such as the effects of internal secretion systems etc., and to conduct re-evaluations of risk assessment when necessary, as well as to strive for more risk reduction

Table IV-3-6 Ground of establishment of dioxins health risk assessment index values(pg/kgBW/day)

	liver hyperplasia nodule	liver cancer
NOAEL (pg/kgBW/day)	1,000	10,000
Uncertainty factor	200	2,000
Details : interspecies difference between rat and human	10	10
Individual difference within human	10	10
Significance of effects	1	10
Possibility of endometriosis	2	2
Health risk assessment index values : (NOAEL/uncertainty factor)	5	5

IV.3.4.3 Exposure assessment

A. Dioxin Residues in the general environment

Concentration of dioxins in sediment and living things (fish) in rivers tend to be a little lower than those of lakes and seas, according to monitoring targeting countrywide rivers, lakes, and the sea undertaken Japan's Environment Agency since 1986. Also, Japan's Environment Agency has been monitoring the atmosphere since 1986, and revealing the trend for concentrations to be highest in residential areas near by industrial factories, lower in middle size urban areas, and lowest in background areas such as mountains.

B. Dioxin exposure situation from the general living environment in Japan

a) Exposure situation from the general living environment

The main routes of dioxins exposure in the general environment in Japan are considered to be (1) food, (2) the atmosphere, (3) water, and (4) soil, and an estimation of the amount of exposure

from each route has been undertaken.

(1) Intake from food (diet)

Dietary intake of dioxins in Japan is estimated to be 163 pgTEQ/person/day (equivalent to 3.26 pgTEQ/kgBW/day) by the market basket method targeting Osaka prefecture. In addition, according dietary intake estimated by the table meal method in 9 prefectures conducted by Japan's Environment Agency, is an average 1.25 pgTEQ/kg/day (0.26 - 2.60 pgTEQ/kg/day). Combing these results these together, dietary dioxins intake in Japan is estimated to be 0.26 - 3.26 pgTEQ/kg/day (**Table IV-3-7**).

Table IV-3-7 Intake of dioxins from food

Country	intake (pgTEQ/kg/day)
Japan (1)	3.26
Japan (2)	1.25 (0.26 - 2.6)
Germany	2.2
Canada	2.3
Netherlands	2.0
USA	0.3 - 3.2
UK	2.1

note : calculated using a standard body weight of
50 kg in Japan, 60 kg in the other countries

(2) Intake form the atmosphere

The following representative atmospheric dioxin concentrations in Japan were assumed on the basis of the results of monitoring in residential areas close to industrial zones, in middle and small sized cities, and background areas from 1990 to 1994, obtained by the Environment Agency, e.g. 0.6, 0.5, and 0.06 pgTEQ/m^3 for big city areas, medium and small size city areas, and background area. Atmospheric dioxin intake of dioxins is shown in **Table IV-3-8** and was calculated by multiplying the above representative concentrations by daily inhaled volume (15 m^3/day) and using a standard body weight of 50 kg.

Table IV-3-8 Estimated intake of dioxins from the atmosphere

	representative concentration (pgTEQ/m^3)	intake (pgTEQ/kgBW/day)
big city area	0.6	0.18
Medium and small size city area	0.5	0.15
Background area	0.06	0.02

note : estimated by using daily inhaled volume of 15 m^3/day, standard body weight of 50 kg

(3) Intake from water

The estimated intake from water is 0.000036 - 0.00048 pgTEQ/kgBW/day or 0.0004 - 0.0012 pgTEQ/kgBW/day of dioxin. From these numbers, it is considered sufficient to estimate the intake of dioxins from water as around 0.001 pgTEQ/kgBW/day.

(4) Intake from soil

Intake from soil is estimated by combining an estimate of intake by this route during childhood and then an estimate of intake for the rest of a person's life time (**Table IV-3-9**)

Table IV-3-9 Intake of dioxins from soil (pgTEQ/kgBW/day)

	oral intake			skin contact intake	total of intake from soil
	Child age	the rest of life time	Total		
City area	0.023	0.060	0.083	0.0013	0.084
Background	0.002	0.006	0.008	0.0001	0.008

(5) Summary of exposure situation of dioxins from the general living environment

The average amount of exposure to dioxins in Japan from the four routes described above is estimated to be around 0.3 - 3.5 pgTEQ/kgBW/day (**Table IV-3-10**).

Table IV-3-10 Average exposure amount of dioxins in the general living environment in Japan

(pgTEQ/kgBW/day)

	Big city area	middle and small size city area	background area
Food	0.26 - 3.26	0.26 - 3.26	0.26 - 3.26
Atmosphere	0.18	0.15	0.02
Water	0.001	0.001	0.001
Soil	0.084	0.084	0.008
Total	0.52 - 3.53	0.53 - 3.50	0.29 - 3.29

b) Exposure situation in 'biased environments'

In order to understand dioxin exposure in Japan in its entirety, in addition to estimating the exposure levels in the general living environment, it is necessary to assume a special, 'biased environment' with higher exposure levels than seen in the general living environment and estimate the difference between these exposure levels. The following two cases were chosen as examples of such biased environments :

- the case assuming that intake from fish is large because of Japanese eating customs
- the case assuming the environment surrounding an incinerator is one of the main sources of

dioxins in Japan

Actual, personal intake of dioxins is considered to have a fairly wide range depending on regional preferences and styles of cuisine. In addition, it is necessary to pay attention that estimation here is based on a certain premises:

(1) Exposure estimation in the case that intake from fish is large

i) Intake of fish

According to a national nutrition survey (1995), Japanese intake of fish and fish products is on average 95.2 g per day, standard deviation 52.0 g. If it is assumed that intake follows a normal distribution, then :

$$\mu + 1.64\sigma = 95.2 + 1.64 \times 52.0 = 180.5 \quad g$$

and about 5 % of people have a fish intake of 180 g per day, i.e. two times the average dietary intake of fish.

ii) Concentration of dioxins in fish

Research results suggest that there are differences in the concentration of dioxins in coastal and inshore fish, deep-water fish and imported fish found for sale in the market place and destined for human consumption, so it is necessary to distinguish both fish species and the source of the fish.

iii) Dioxin intake from fish

Representitive concentrations of dioxins in coastal and inshore fish are 0.90 pgTEQ/g. Representitive concentrations in deep-sea fish are 0.1 pgTEQ/g and 0.08 pgTEQ/g for imported fish.

(2) Intake of dioxins in the case that intake from fish is large

This estimation is performed by using the two assumptions shown below. However, whether such intake is continuous is dependent on the dining habits of the individual, and actual personal intake can be smaller than this evaluation.

i) in the case that fish intake is two times of average

It is assumed that there are 5 % of people who eat twice the average intake of fish, and in this case the estimate also assumes that these people don't eat meat or eggs as animal protein.

- Based on exposure research in Osaka using the market basket method, intake from fish is assumed to be 105 pgTEQ/day, or 4.2 pgTEQ/kgBW/day (2 x 105 pgTEQ/day ÷ 50 kg).
- It is assumed that the average intake of dioxins from food found in a survey conducted by the Environment Agency (1.25 pgTEQ/kgBW/day) originates in fish. Based on this, the average intake of dioxins from food is 1.27 pgTEQ/kgBW/day (2 x 1.25 pgTEQ/kgBW/day x 0.508).

- By using the above averages, dioxin intake from fish is 2.74 pgTEQ/kgBW/day [2 x (2.1 + 0.64) ÷ 2].

ii) in the case of intake of average amount fish from mainly coastal area

In this case, dioxin concentration in fish is 0.9 pgTEQ/g, and the average intake of fish is 95.2 g, then the average intake of dioxins from fish is 1.71 pgTEQ/kgBW/day (0.9 pgTEQ/g x 95.2 g/day ÷ 50 kg). There has been little data on dioxin intake from fish in Japan, and the few estimates of intake from fish have a wide range, but putting them together, the average intake of dioxins from food becomes 2.74 pgTEQ/kgBW/day, and possibility of dioxins intake from fish is estimated as around 1.28 - 4.2 pgTEQ/kgBW/day. In addition, including dioxin intake from other routes, dioxin intake is estimated as 3.59 pgTEQ/kgBW/day (1.90 - 5.28 pgTEQ/kgBW/day) in the large urban area (**Table IV-3-11**).

Table IV-3-11 Dioxins intake when the intake from fish is large (pgTEQ/kgBW/day)

	large urban areas (big cities)	medium and small cities	background (rural) areas
Food	3.32 (1.63 - 5.01)	3.32 (1.63 - 5.01)	3.32 (1.63 - 5.01)
Atmosphere	0.18	0.15	0.02
Water	0.001	0.001	0.001
Soil	0.0084	0.084	0.008
Total	3.59 (1.90 - 5.28)	3.56 (1.87 - 5.25)	3.35 (1.66 - 5.04)

Assumption : dietary intake of fish is twice the average amount of fish from inland sea and bay

(3) Exposure around incinerators

In this section, in order to understand exposure around incinerators, the largest yearly average concentration of dioxins which reaches the is predicted by calculating the diffusion of gas fumes from incinerator chimneys using modelling.

i) classification of incinerators and prediction of diffusion concentration

In order to characterise incinerators, incinerators are classified using 47 criteria, such as furnace type, cooling method, dioxin strategies, methods for treating gas fumes, height of chimneys etc. The maximum yearly average landed concentrations of dioxins originated in gas fumes from chimneys were predicted by atmosphere diffusion model for each classification. Estimates were conducted by assuming discharge of dioxins at concentrations two standard deviations higher than the average, according to the discharge concentration distribution current incinerators and the average discharge concentrations of dioxins. In these cases, incinerators were assumed to represent an average facility, and a composite of a mechanised batch system and independent chimney.

ii) Results of prediction of diffusion concentrations

For facilities which have average discharge concentrations, the maximum, yearly average landed concentrations of dioxins were predicted as 0.01 - 0.8 pgTEQ/kgBW/day incorporating measurements of dioxins in gas fumes, and 0.2 - 1.9 pgTEQ/kgBW/day without. For facilities which have higher than average discharge concentrations, the maximum yearly average landed concentration was predicted as about 3 pgTEQ/kgBW/day.

iii) Establishment of environmental concentration around incinerators

Dioxins concentrations in the atmosphere around incinerators were established as 3 - 4 pgTEQ/m^3 from the results of predictions of diffusion concentrations, and concentrations in the atmosphere in large cities. Atmospheric fall out was determined by the amount of deposition at the point of the maximum landed concentration. Concentrations in soil were obtained from actual data obtained from samples taken around incinerators used for industrial wastes.

iv) Total intake of dioxins around incinerators

Total intake of dioxins around incinerators was predicted by combining the amount of atmospheric deposition (100 ngTEQ/m^3/year), concentrations in the atmosphere (3 - 4 pgTEQ/$m)^3$, and concentration in soil (150 pgTEQ/g) s shown as **Table IV-3-12**.

In this section, the above two cases of special 'biased environments' were chosen as examples, but there is considered to be a wide range of actual personal exposures depending on differences in regional conditions and eating habits etc. These predictions are made using incomplete data for dioxins in the general environment and food etc. available from exposure assessment, and accumulation of data in future is necessary.

Table IV-3-12 Dioxins intake around incinerators

	intake (pgTEQ/kgBW/day)
food	0.26 - 3.26
atmosphere	0.9 - 1.2
water	0.001
soil	0.63
total	1.79 - 5.09

IV.3.4.4 Risk judgement

In Japan, 10 pgTEQ/kgBW/day was used as the TDI for dioxins, and 5 pgTEQ/kgBW/day as the health risk assessment index value. Then, from exposure assessment, an exposure of 0.3 - 3.5 pgTEQ/kgBW/day in the general living environment was estimated. The average amount of

exposure in groups which have twice the average intake of fish was assumed to be 3.6 pgTEQ/kgBW/day (1.0 - 5.3 pgTEQ/kgBW/day). The average amount of exposure in groups living around incinerators was estimated as 1.8 - 5.1 pgTEQ/kgBW/day. Putting all this together, the following can be concluded about the risk posed by dioxins in Japan:

- The possibility of health effects from general environmental exposure is considered to be very small at this point in time, because estimated exposure values are lower that health risk assessment index values. However, it is thought desirable to attempt to reduce environmental concentration of dioxins from this point forward in order to secure higher long term safety, because present exposure levels cannot be said to be low enough when compared to assessment index values.
- In order to decrease health risks in the future, there must be an attempt to reduce environmental concentrations of dioxins, because it is possible that estimated values of the amount of exposure will be the same as, or more than, the health risk assessment index values under conditions of especially high exposure..

The following views were not mentioned in this section's risk assessment of dioxins : A. mother - child transfer of dioxins through breast milk, and B. coplanar PCBs.

A. Intake from breast milk

Dioxins are secreted into milk. Dioxin concentrations in breast milk are approximately the same in the developed countries, including Japan. However, the WHO and many other nutrition experts keep promoting breast milk for infant nutrition because mother's milk has been shown to have clear advantages for infant health and growth. Since continued breast feeding is appropriate, proper measures to find the sources of contamination should be undertaken to secure the safety of mother's milk now and in the future.

B. Coplanar PCBs

It is difficult to conduct accurate exposure assessments of coplanar PCB because there is not enough data about environmental concentration etc., and dioxin toxicity equivalent factors have not evaluated. The toxicity of coplanar PCB has a similar mechanism to that of dioxins, so the human health risk through exposure to these compounds must be assessed, and to do this there must be more research and more accumulated knowledge of the toxicity of coplanar PCBs.

IV.3.5 Revision of the Tolerable Daily Intake (TDI) for dioxins

In 1996, the Japanese government set the TDI (Tolerable Daily Intake) of dioxins (PCDD/Fs : polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans) at 10 pg / kg BW / day 2,3,7,8-TeCDD after the above mentioned procedure (**IV.3.3**). At the same time, "risk evaluation index values", which are defined as "the level which is desirable to maintain positively", were set at 5 pg/kgBW/day. Thereafter, in May 1998, the WHO European Centre of Environment and Health and IPCS (the International Programme on Chemical Safety) investigated published information on the toxicity of dioxins, and suggested that the TDI of PCDD/Fs should be 1 - 4 pg/kgBW/day, including 12 kinds of coplanar PCB (Co-PCBs). In response to this, in June 1999, the Japanese Government began to revise the TDI for dioxins (PCDD/FS and Co-PCBs), and decided on a value of less than 4 pg/kgBW/day. This change basically followed the WHO's suggestion. However, one feature of this process is that TDIs are determined not NOAEL or LOAEL which are obtained by animal toxicity tests, but body burdens. Therefore, the process of TDI calculation described in this section.

IV.3.5.1 Basic concept

The tolerable daily intake (TDI) is the amount of chemicals ingested per day which it is judged will not cause harmful health effects to appear even when humans ingest that amount for life.

After considering the movement of dioxins in the body, toxicity mechanisms etc., it is appropriate to base the calculation of TDI on the following concepts (**A.** to **D.**) based on the same criteria as the ones the WHO meeting adopted.

A. Judgment that there is no genetic harm

Dioxins are considered not to cause any direct genetic harm, and, therefore, the threshold level becomes the boundary of suspected harmful health effects. Therefore, the method applies an uncertainty coefficient into the NOAEL or LOAEL for calculation of the TDI.

B. Aiming at a body burden

For substances which have a high accumulation character but show big differences in the degree of accumulation between species, such as dioxins, it is appropriate to aim at a body burden rather than an amount of intake per day in order to investigate relations between the substances and the effects.

When small amounts of substances which have a high accumulation character have been taken continuously for long time, at first the accumulated amount of material increases because more is absorbed than is metabolised and discharged. However, as the amount accumulated increases, the amount of metabolism and discharge also increases, and the amount of material which exists in the body (the body load) reaches an equilibrium at a definite level corresponding to the amount of intake.

In general, the appearance of toxicity attributable to the chemical substance is related to the amount of material which exists in the body. However, it is important to know how much chemical substance is continuously ingested or absorbed by humans when the body load reaches the level which causes toxicity, in order to evaluate the toxicity of substances which have high accumulation characteristics.

Dioxins also show big differences in the rate of disappearance (half life) between species. Therefore, when applying results gained from toxicity tests to human beings, it is appropriate to aim not at dose, but body load, to determine the body load which causes health effects in test animals, and to determine how much amount is continuously ingested to reach the body load for human beings.

C. Evaluation of test data

For each toxicity test in which the toxic response at the lowest body load, after considering the toxicological significance of the reaction, is aimed at an evaluation index, dose dependency, reliability and repeatability of the test etc. becomes a TDI calculation object.

For dioxins, there are many reports of toxicity test results. Some of them judge that the responses which appear in animals has no toxicological significance, and some are tests whose reliability and repeatability are not good enough, even if the response itself has toxicity significance. These test results are not appropriate for use a baseline data from which to conduct quantitative evaluation of toxicity. Therefore, it is necessary to discuss carefully which test results should be chosen in order to adopted as the basis of a TDI calculation.

D. Set up of uncertainty coefficient

When the TDI of humans is calculated from the results of toxicity tests, factors such as species differences between animals and humans, individual differences in sensitivity to test substances between humans, and the reliability and propriety of toxicity tests etc. carry much uncertainty and have big effects on the calculated values. Therefore, during calculation, each factor must be carefully discussed, an appropriate coefficient (the uncertainty coefficient) set up, and methods

which compensate for the uncertainty chosen.

In the case of substances with extremely varied effects on living organisms, and where the appearance of such effects shows big difference between species and lineage, such as dioxins, the significance of the uncertainty coefficient is very important for toxicity evaluation.

In general, the uncertainty coefficient for species differences and individual differences are set up based on knowledge about the movement in the body, and the mechanisms of action. Test conditions, dose dependency and the toxicological significance of the effects which are used for evaluation etc. are also important factors for the reliability and propriety of toxicity tests.

IV.3.5.2 Body load in each toxicity test

Body burdens have been determined from 2,3,7,8-TCDD toxicity tests by collecting data from those extremely low doses which cause toxic reaction. Since such toxicity test data barely have appropriate NOAELs, LOAEL data were used instead. Also, when reliable values are available for body burden, such information was adopted, but otherwise calculated values based on estimates from literature knowledge were adopted.

IV.3.5.3 Animal body load which becomes calculation basis of TDI

Factors such as the adequacy of the results of toxicity tests which investigated the effects on derivatisation of drug metabolic enzyme (CYP1A1), the composition of lymphocyte, chlordane, immune toxicity, sex organ system (spermatozoon production, endometritis, sex organ form), and learning ability were discussed as the basis for TDI calculation by considering toxicological significance, dose dependency, reliability and reproducibility of tests.

IV.3.5.4 Human body load

There are no reports of systematic research into the relationship between species difference and body burden upon appearance of the effects of dioxin of toxicity. However, generalising the results of existing toxicity tests and epidemiological research, it is judged that there is no big difference between the body load which causes toxic effects in human and animals.

IV.3.5.5 Calculation of daily human intake

The same calculation and formula as that adopted by the WHO specialist meeting is used to estimate the average daily intake required for humans to reach their lifetime exposure body load.

IV.3.5.6 Determination of uncertainty coefficient

It is necessary to apply an uncertainty coefficient to calculate human TDI based on LOAEL for human which have been estimated from toxicity data in order to compensate for the inherent uncertainty of the data. The following factors, some of which are the same as those used by the WHO specialist meeting, were considered for the coefficient :

1. Use LOAEL instead of NOAEL as the baseline values for TDI calculation.
2. It is not necessary to consider species differences originating from transport in the body (see IV.3.5.4) because body burden is used at the time of calculation of the least toxic amount for humans.
3. There is no clear knowledge that human are more sensitive to dioxins than animals. Rather there is data which show that human are less sensitive. For example, Ah receptor affinity research.
4. Lack of knowledge about individual differences in the rate of appearance of toxic effects in humans.
5. Lack of knowledge about the half-life of each dioxin congener in humans.

IV.3.5.7 Determination of TDI

A. Selection of body load on which TDI calculation is based

According to tests which have clearly evaluated toxicity effects, the relationship between body load and the appearance of toxic effects in each toxicity test suggest that the value of the lowest appearance body load will be around 86 ng/kg.

B. Report of WHO (European Region Secretariat) specialist meeting

A specialist meeting of the WHO European Centre suggested TDI values within 1-4 pg TEQ/kg/day, but did not think that some reported tiny effects were obviously bad effects even when these tiny effects happened in residents of developed countries. Also, they considered 4 pg TEQ/kg/day as a tolerable daily intake at present, because confirmed effects can be related to other compounds than dioxins. However, they did suggest it appropriate to have a final goal of decreasing human intake levels to less than 1 pgTEQ/kg/day. Japan accepted this concept.

C. Conclusion

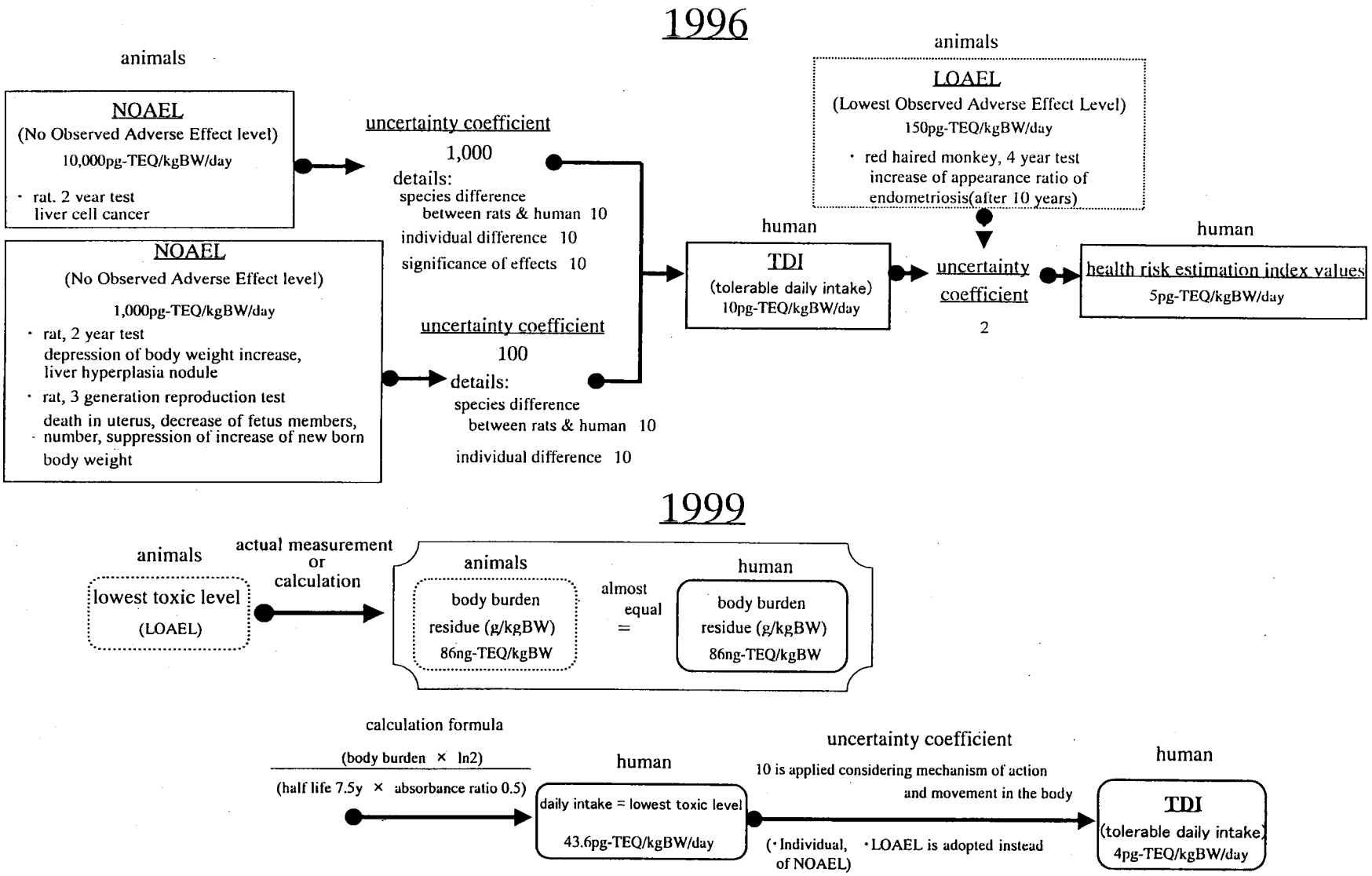
Although there are some unresolved aspects concerning the human health effects of dioxins, at present the TDI for dioxins had been set at 4 pgTEQ/kg/day (including coplanar PCB) based on calculations incorporating an uncertainty coefficient, a human daily intake of 10 to 43.6 pg/kg/day which corresponds to body load value, and 86 ng/kg as 2,3,7,8-TCDD. However, there are some known small negative effects at body loads less than 86 ng/kg, and, therefore, it is important to further toxicity investigations to determine the significance of these toxicological observations.

IV.3.5.8 Difference from former TDI calculation methods

Comparison between the 1996 and 1999 TDI calculation methods is shown in **Figure IV-3-3**. Although the 1996 version determines human TDI directly by applying an uncertainty coefficient to the non-toxic concentrations determined in toxicity tests, the 1999 version does not use the amounts administered in toxicity tests, but actual body loads as the basis of TDI calculations.

Usually, when a human TDI is determined from the results of toxicity tests, a standard uncertainty coefficient of 100 has been applied. During a recent risk assessment risk, scientific knowledge of the transport and mechanisms of action of dioxins in the body were introduced in order to set uncertainty coefficients based on species and individual differences, and these methods of estimating values appropriate for human application have come to be used. At that time, an uncertainty coefficient of 10 was set.

Again, usually TDI evaluation targeted results of long-term, continuous administration toxicity tests. However, such evaluation is based on the premise that dioxin toxicity is mainly manifested through combination with the Ah receptor, and it thus became possible to apply the results of single and short term administrations to animals to long term human exposure to trace amounts by using the concept of body loads. Therefore, highly sensitive, multiple health effect indexes, such as reproductive toxicity tests etc., can be considered.



FigureIV-3-3 Comparison of TDI calculation methods for dioxins

IV.3.5.9 Significance and notes on TDI

The general significance of TDIs is that TDIs are values calculated with the intention of aiming for a maximum daily intake without manifestation of negative health effects in the case of continuous intake for life. Therefore, there is no suggestion of harm to health if the long term average intake is within the TDI, even if intake temporally exceeds the TDI by a small amount.

The new TDI aims to minimise effects of exposure in early life stages (embryo / fetus) which are thought the most sensitive to dioxin toxicity tests. Therefore, for evaluation of human beings as a whole, the new TDI can be regarded as being on the safe side. In this respect, effects such as carcinogenicity appear as a result of higher exposure. Sensitivity differences between humans and animals, and individual differences are included by applying an uncertainty coefficients to the results of toxicity tests.

Exposure to dioxins is mostly from food. Individual contamination will vary depending on the type of food consumed. However, nutritionally it is important to eat a balanced diet. In addition, the benefits of breast feeding for infants are such that breast feeding should be encouraged, while scientists continue to research the effects of dioxin exposure through breast feeding on infants. Such conclusions were also reached by the WHO specialist meeting.

At present, the average Japanese daily intake is about 2.6 pgTEQ/kg/day. Exposure seems to be decreasing as dioxin concentration in breast milk is decreasing.

The current human exposure to dioxins in Japan cannot be said to be low enough when compared to the new TDI. Therefore, environmental discharges must be reduced in order to reduce concentrations of dioxins in food chains, and reduce human body loads. Since dioxins are not valuable chemicals produced commercially for the good of humanity, and at the very least they are non-beneficial, and possibly harmful, to all living things, it is obvious that reducing intake as much as possible is desirable for the benefit of all in the future.

IV.4 Ecological risk assessment

In the nature there are many, diverse living things and in adapting themselves to survive in their environment, they form ecosystems made up of many interdependent relationships which in turn contribute towards keeping an active balance of material and energy circulation. When chemicals invade in such systems, they may cause direct toxic effects on living things or cause indirect toxicity by changing the environment. When the effect on an organism exceeds its natural range of tolerance, interdependent relationships between organisms become irregular, at which point the ecological effects of the chemical begins to be observed . Ecological risk assessment of chemicals is an operation used to estimate the likelihood at which more than one chemical in the environment produces adverse effects on organisms and ecosystems. The results of ecological risk assessment are used as a standard for chemical management.

Ecological risk assessment is still in the process of being developed compared with human health risk assessment because of the many difficulties faced, such as species diversity, diversity of effects, differences in sensitivity, and differences in perceived value e.g. about which living things should be protected. The US EPA provided some general principles for ecological risk assessment in its "Guide to Ecological Risk Assessment" in 1996, and the EU describes methods of ecological risk assessment in the "environmental assessment " section of its "manual of new and present chemical risk assessment technique" published in 1997. In addition, the OECD strengthened the scientific technology basis of ecological risk assessment in the safety assessment program of high production volume chemicals (HPVC) and established ways for its active use. In this section, ecological risk assessment methods based on the methods of the OECD HPVC project are described since they have the most satisfactory results.

IV.4.1 Outline of ecological risk assessment of OECD HPVC project

The HPVC of OECD are a group of chemicals which produced in volumes greater than 1,000 ton in more than two countries, or more than 10,000 ton in one country. The project is based on the judgement that, in order to assess the environmental effects caused by current HPVC chemicals, it is most efficient to co-operate internationally to collect and evaluate data. This project has established a screening information data set (SIDS) for early risk assessment, supplementing patchy HPVC data, and then judges the necessity of additional investigation and tests based on each chemical's SIDS (**Table IV-4-1**).

Table IV-4-1 SIDS items necessary for early assessment

General information	Chemical Abstract registry No. (CAS No.), compound name, chemical structure
Source	production volume, use
Physicochemical characters	melting point, boiling point, density (specific gravity), vapour pressure, octanol-water partition coefficient (P_{ow}), water solubility, pH and pKa values, oxidation reduction potential
Environmental movement	photodegradation, degradation in water, biodegradation, Henry's law constant, environmental monitoring data
Ecological toxicity	fish acute toxicity, daphnia acute toxicity, algal growth inhibition, daphnia reproduction inhibition*, other toxicity (soil organisms, plants, birds)* →* : the necessity of the test depends on the judged degree of exposure
Toxicity	acute oral, inhalation, dermal toxicity, 28 days repeat administration toxicity, hereditary toxicity (in vitro, in vivo), reproduction toxicity, teratogenesis

This evaluation of early stage ecological effects of HPVCs on the hydrosphere by SIDS is used to clarify the risk to ecosystems by comparing the maximum tolerable concentration (MTC) and predicted environmental concentration (PEC). Ecological risk assessment based on results of environmental monitoring becomes possible by substituting the PEC with actual measurement data.

The MTC of ecosystem of the hydrosphere is considered to be the maximum chemical concentrations which don't appear to produce intolerable harmful effects by exposure, and the OECD supposes that when 95% of organisms are protected, hydrosphere ecosystems are preserved, i.e. safe. However effects on endangered species and precious species are considered individually.

The MTC of chemicals in a hydrosphere ecosystem is assessed by a three-stage process: initial stage, refined stage and comprehensive stage depending on available information type and quantity. **Figure IV-4-1** shows the flow chart.

At first, the data available for ecological risk assessment has to be checked and evaluated. At this point ,important factors are a chemical's octanol-water partition coefficient (P_{ow}), bioconcentration character, and effects on aquatic organisms.

P_{ow} should be evaluated carefully because it is very important to the initial stage of risk assessment. For example, P_{ow} obtained by the shaking flask method is not appropriate for measurement of non-polar chemicals ($P_{ow} > 5$), so the slow stirring method or generator column method should be used. Also, P_{ow} is not appropriate for surfactants, polymers, inorganics, and organometallic compounds.

Bioconcentration character is an index of indirect effects, obtained by experiments or

quantitative structure activity relationships (QSAR). However, QSARs often cannot predict concentrations of chemicals which are non-polar under outdoor conditions. When there are more than one bioconcentration factor (BCF) for some species, the geometric mean for the species should be used, but experimental conditions should be considered as well. When there is a BCF for more than two species, use the one for the most nutrient intensive lifestage. BCFs for algae, daphnia, and fish should be treated separately.

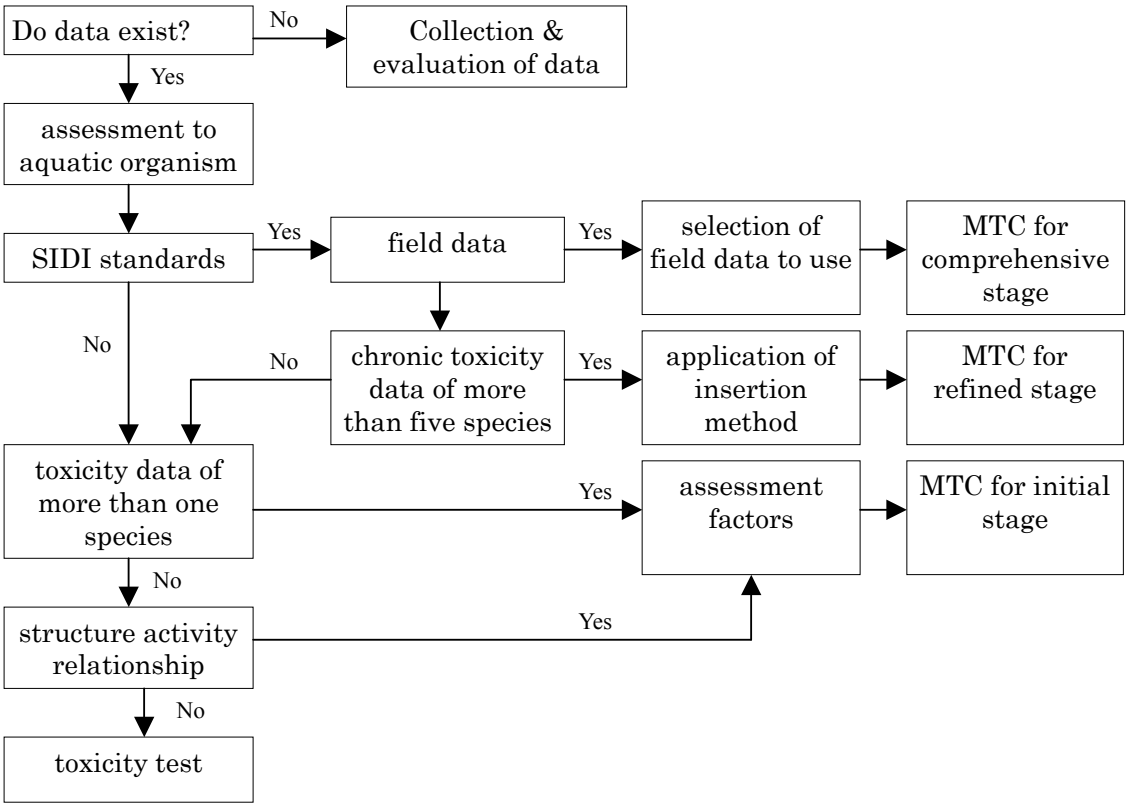


Figure IV-4-1 Flow chart for calculation of maximum tolerable capacity (MTC)

The results of chronic toxicity tests are needed for chemicals which have a high BCF. A 96 hr acute toxicity test is not long enough for these compounds. Know the solubility of the target compounds in water, solubility limits and effective concentrations.

Interpretation of toxicity data is important, for example, measurement / set-up concentration, response of control group, use of high sensitivity species, and water quality values. Endpoints such as survival, growth, reproduction should be paid more attention than other endpoints such as biochemical parameters. If a chronic toxicity test wasn't conducted for most sensitive species determined by acute toxicity testing, it is necessary to pay attention to the kinds of experimental animals used.

When there is a range of toxicity data for one species, the following points should be considered.

- If toxicity data is collected over the same period as the effect parameter (endpoint), use geometric parameter.
- When effect parameter and exposure period are different for one species, use the lowest values from the longest experiments after considering the importance of both endpoint and exposure period.
- Data used for insertion method are limited only for NOEC values and geometric mean MATC values [MATC = (NOEC x LOEC)$^{1/2}$]. For a chronic toxicity test which reports only the lowest effect concentration (LOEC), if the values are converted to estimated NOEC values properly, they can be adopted. For example, NOEC = LOEC / 2 for one particular case can be used. Also regression formula for estimation can be used.

At the initial stage, generally short term (acute) toxicity test for primary producers (algae), primary consumer (daphnia), and predators (fish) is needed. At more refined stages, long term (chronic) toxicity, semichronic toxicity tests, and at the comprehensive stage, diverse species mixture test and data from field observations. The initial evaluation based on SIDS applies an evaluation factor. If there is no toxicity data, or only data from one species, or the data is unreliable, predicted values by QSAR can be used if the situation warrants it.

For compounds where data is available which exceeds the range of SIDS, higher level evaluation can be conducted. If chronic NOEC for more than five different species is available, the insertion method which considers sensitivity of other species can be applied. Also, if data from short term multi species tests and (semi) field tests, including long term ecosystem observations, are available, comprehensive evaluation becomes possible by combination with chronic toxicity data. Evaluation factors in this case are judged depending on the situation. Indirect effects on birds and mammals and effects on benthic organisms can be considered depending on the use and characters of chemicals.

Thus, ecological risk assessment becomes possible by comparing “estimated non-effective concentration” which are basically expressed as PNEC and MTC etc. and “data from environmental monitoring”. Therefore, according to the above mentioned processes, the kinds and meaning of ecological toxicity tests which are necessary for the calculation of estimated non-effective concentration, the use of toxicity data and risk assessment method are described as follows:

IV.4.2 Ecological effect tests

IV.4.2.1 Representative values of toxicity

The results of ecological effect tests are eventually collated statistically as representative values such as LC_{50} (lethal concentration 50 %), LD_{50} (lethal dose 50 %), EC_{50} (effect concentration 50 %), ED_{50} (effect dose 50 %), TC_{50} (tumour concentration 50 %), TD_{50} (tumour dose 50 %). These representitive values become important indexes for ecological risk assessment. The capital letters, L, E, and T, of these description indicate a toxicity endpoint : L is short for the lethal of lethal toxicity, E, observed effects (endpoints) such as abnormality in appearance, behavioural change, biochemical pathological change, growth inhibition, reproductive inhibition, increase in rate of deformity, etc; T is used for appearance of tumours as the endpoint. The second capital letter indicates the exposure routes : C means concentration in air or water, D an oral or dermal dose. Since ecological effect tests have been mainly conducted for aquatic organisms, more data is available on concentrations than direct doses. The letters of the end indicate ratio of the observed number of endpoints against a given sample number, shown as a percentage. Therefore, LC_{50} means half of a given sample will die at the stated concentration, and LC_{10} indicates an exposure concentration which causes mortality in 10 % of the sample. By the way, E often means not only hazardous effects, but also harmless or useful effects, for example ED_{50} is frequently used for judging the effects of animal medicines.

In ecological effect tests, the greatest concentration of a chemical which doesn't cause an effect with a significant difference from the control group is called the no observed effect concentration(NOEC). The NOEC is an important risk assessment index, and is calculated from experimental data statistically (multiple comparison test at 5% risk rate). The no observed effect level (NOEL) is the maximum amount of toxicant that does not produce an effect, and may be calculated by converting the NOEC to a level, and is used for both exposure concentration and intake. The lowest concentration which causes an effect is the lowest observed effect concentration (LOEC).

IV.4.2.2 OECD ecological effect test methods

The OECD recommends 11 test methods in its ecological effect test guidelines, revising and adding to them as required, and ecological risk is assessed from the results of such tests either comprehensively or step by step. Of the tests, the algal growth inhibition test, the daphnia acute toxicity test, and the fish acute toxicity test are designated as 'minimum premarketing sets of data'

(MPD), and the minimum toxicity data necessary for initial stage screening. The Japanese Environment Agency conducts five tests - algal growth inhibition test, daphnia reproduction test, fish acute toxicity test, plant growth test, and earthworms acute toxicity test.

A. Algal growth inhibition test (201)

a) Purpose and meaning

Algae are the primary producers in the hydrosphere's food network. Plants which produce food and oxygen are the most basic requirements for consumers. Extreme increase and decrease in algal growth caused by chemicals will affect organisms at higher trophic levels in natural ecosystems. This test can investigate the effects of chemicals on growth and reproduction of single celled green algae for several generations under standard experimental conditions. However, it is often not appropriate to extrapolate the results of this test to effects on other primary producers.

b) Experimental method and results

Algae species used in this test are *Selenastrum capricornutum*, *Scenedesmus subspicatus*, and *Chlerella vulgaris*. Standard culture techniques are used. Under continuous lighting, the algae are exposed to the target compound for 96 hours at 21 - 25 ± 2 °C, and the growth of the algae is measured (total organism or cell numbers) at 24, 48, 72, and 96 hours after the beginning of the experiment. By drawing a growth curve from the data obtained, and comparing the area underneath the growth curve, or the growth rate, with that of the control, inhibition rates such as the EC_{50} and NOEC can be calculated.

B. Daphnia acute toxicity test (202 Part I)

a) Purpose and meaning

Daphnia are important primary consumers in the hydrosphere ecosystem, and useful for initial stage risk assessment because they are sensitive to many toxic substances, and because their response is similar to other aquatic invertebrates. This test also plays a role as a preliminary, or concentration range-finding test for the next step (reproduction test : Part II). The daphnia test is an economical method which gives useful information for risk assessment or planning of supplementary test.

b) Experimental method and results

The daphnia species used in this test is *Daphnia magma* or other daphnia, cultured in the standard culture medium within 24 hours after birth. The daphnia are exposed to target

compounds for 24 hours (48 hours is also OK) without feeding (at 18 - 22 ± 1 °C). After 24 or 48 hours observation, the number of mobile / immobile daphnia is counted Swimming inhibition is defined as being when daphnia doesn't move after mechanical stimulation or gentle shaking. The inhibition rate for each concentration is calculated, and the EC_{50} and the lowest concentration which inhibits swimming in 100 % of daphnia is calculated

C. Daphnia reproduction test (202 Part II)

a) Purpose and meaning

Daphnia are important primary consumers in the hydrosphere ecosystem, and useful for initial stage risk assessment because they are sensitive to many toxic substances, and because their response is similar to other aquatic invertebrates. This test uses the results of the daphnia acute toxicity test (Part I), and is considered to belong to the category of long-term (chronic) toxicity tests because it covers the life cycle of daphnia, the and the standard effects assessed, i.e. endpoints, give useful information economically for risk assessment or the planning of supplementary experiments.

b) Experimental method and results

The daphnia species used this test are the same as used in daphnia acute toxicity tests (Part I). Lighting cycle, hours light, and 8 hours dark. The daphnia are exposed to the chemical for at least 14 days at 18 - 22 ± °C. The experiment is conducted in flow-through systems, and total exchange of water and observation are done at least every 48 hours. Observations with the naked eye include such things as life/death of parent, existence of eggs in the incubator male, dormant eggs, parent size, number of babies, etc. The rate of inhibition of reproduction for each concentration is calculated, and the EC_{50} and LC_{50} calculated, The NOEC is obtained by statistically comparing the number of babies in the treatments against those in the control.

D. Fish acute toxicity test (203 revised)

a) Purpose and meaning

Fish are high level consumers in hydrosphere ecosystems. Fish acute toxicity tests can give index of concentration-response relationships, but cannot give information about target organ toxicity mechanism because pathological dissection is not conducted.

b) Experimental method and results

Fish typically used are Zebra fish, *Brachydanio rerio*, Flathead minnow, *Pimephales promelas*,

Carp, *Cyprinus carpio*, Red killifish, *Oryzias latipes*, Guppy, *Poecillia reticulata*, Bluegill, *Lepomis macrochirus*, and Rainbow trout. Tests may be conducted in static or flow-through systems. Lighting is 12 - 16 hours/day, water temperature is appropriate for the test species ± 2 °C, and exposure to target chemical is for 96 hours without feeding. Mortality is observed at 24, 48, 72, and 96 hours after the test begins. The mortality rate for each concentration is calculated, and LC_{50}, the lowest concentration that causes 100% mortality, and NOEC calculated

E. Fish prolonged toxicity test (204)

a) Purpose and meaning

This test is used when longer observation than are possible with acute tests are more appropriate to gather the information needed.

b) Experimental method and results

The same fish species used in acute toxicity tests may also be used for longer term tests, i.e. Zebra fish, Flathead minnow, Carp, Red killifish, Guppy, Bluegill, and Rainbow trout. Aqueous concentrations of the test chemical are set such that lethal and other sub-lethal effects can be determined, and NOEC values calculated. Tests may be conducted in static or flow-through systems. Lighting is 12 - 16 hours/day, water temperature is appropriate for the test species ± 2 °C, and exposure to target chemical is for 14 days. Mortality is observed every day, and other effects as appropriate e.g. appearance, abnormal swimming behaviour, response against stimulation, and feeding may be checked daily, body length and body weight changes checked at the end of the exposure period. If necessary, the observation period can be extended for a further 1 - 2 weeks. The NOEC is determine after calculating the mortality rate for each concentration.

F. Bird acute toxicity test (205)

a) Purpose and meaning

After feeding the birds the target compound for five days, determine the chemical concentration in the food causes 50 % death within three or more recovery terms.

b) Experimental method and results

Use juveniles of Mallard, Quail (Korin-uzura), Pigeon, Common Quail, Pheasant (Korai-kiji), and Partridge. Feeding conditions depends on the bird species, but in general enclosures (bird cages), temperature, humidity, and lighting should be suitable for keeping them. Feed the test groups food containing target chemical by for five days,. Allow the birds to feed as much, or as

little, as they want. Then feed chemical-free for at least three days. If effects continue, extend the period, and observe toxic symptoms, abnormal behaviour, death, body weight, and feeding amount. The mortality rate for each concentration and LC_{50} calculated.

G. Avian reproduction test (206)

a) Purpose and meaning

To investigate the concentrations of target chemical which affects avian reproduction by comparing with control.

b) Experimental method and results

Adult birds of Mallard, Quail (Korin-uzura), Common Quail etc. Enclosures depend on the species and age of the birds, and the temperature (22 ± 2 °C), humidity, and lighting should be suitable for keeping them. Raise either a pair of birds, or one male and two females as a group in the enclosures. Experiments start when the birds are first given food containing the target chemical. Allow the birds to feed freely throughout the experimental period. When egg laying begins, collect the eggs every day and store them in an incubator to hatch them. Don't incubate obviously damaged eggs. Measure the thickness of the egg shell for a number of eggs from each treatment. Compare mortality rates, body weight, amount of feeding, pathological observation, number of eggs laid, number of damaged eggs, thickness of egg shell, survival ability, incubation rate of parent birds, and survival rate, body weight, feeding amount of juvenile birds, with the control groups, then determine the statistically significant NOEC and effective concentrations.

H. Earthworm acute toxicity test(207)

a) Purpose and meaning

This basic, short term exposure test using terrestrial organisms uses soil as the transport medium for pollutants. There are two tests, one uses an artificial soil, the other simplified test is a filter paper feeding toxicity test.

b) Experimental method and results

Use *Eisenia foetida* which are at least 2 months old. For the filter paper feeding toxicity test, expose the worms to the target chemical in the dark at 20 ± 2 °C for 48 hours (or 72 hours) without feeding, and observe mortality rate after the exposure ceases. For the artificial soil test, expose the worms to the target chemical under continuous lighting at 20 ± 2 °C for 14 days without feeding, and observe fate of earthworms on days 7 and 14. Calculate the rate of inhibition for each

concentration, and determine the LC_{50}, the lowest concentration which causes 100 % mortality, and the lowest concentration in which 100 % survive.

I. Plants growth test (208)

a) Purpose and meaning

This test is used to assess the effects of solid or liquid target chemicals on germination and early stage growth of various land plants in soil which has been treated once with the target chemical.

b) Experimental method and results

Plants used for the test are divided into the following three categories, i) rice plant, wheat, etc. ii) rape, radish, turnip, etc. iii) pea, lettuce, koroha, etc. Choose at least one species from each category. Use soil which has been sifted through a 0.5 mm sieve, has a carbon content of less than 1.5 % (organic matter content 3 %), contains 10 - 20 % of particles less than 20 μm, and whose pH is adjusted between 5.0 - 7.5. Within 24 hours of the target chemical being mixed into the soil, plant more than five seeds of the same size, and raise under appropriate conditions of temperature, humidity, and lighting, and watering. Harvest the plants no earlier than 14 days after 50 % of control group has germinated, and calculate the rate of germination for each concentration and the average weight per plant. Calculate the rate of inhibition for each concentration, and determine the EC_{50} (growth) and LC_{50} (germination).

J. Activated sludge respiration inhibition test (209)

a) Purpose and meaning

This tests monitors the effect of activated sludge on decomposers in the environment. It is a useful test to monitor the effects on sewage treatment facilities because it is not sensitive to even highly toxic chemicals. This test determines chemicals which cause harmful effects to aerobic microorganisms in sewage treatment facilities, and provides a quick screening method to determine chemical concentrations which do not inhibit biodegradation tests.

b) Experimental method and results

Use 3,5-dichlorophenol as a standard compound. The EC_{50} (3 hours) of this compound is 5 - 30 mg/L. The activated sludge used for the test is collected from a sewage treatment facility which mainly treats domestic sewage, and made up to 4 g MLSS/L after washing. Control1 : add 16 mL of artificial sewage before start of the test, add 200 mL of activated sludge and aerate by Pasteur

pipette (0.5 - 1.0 L/min). Prepare test concentrations every 15 minutes, and finally, make Control 2 in the same way as Control 1. After three hours, measure the respiration rate of Control 1 by using a dissolved oxygen meter for 10 minutes (Rc_1). Measure the respiration rate of each test concentration every 15 minutes (Rs), make each feeding time 3 hours. Finally, measure Control 2 (Rc_2). Feeding time can be 30 minutes. Calculate respiration rates from a locus of linear part within about 6.5 - 2.5 mg/L of dissolved oxygen, and determine inhibition rate from the formula [(1 - (2Rs) / (Rc_1 + Rc_2)].

K. Fish early life stage test (210)

a) Purpose and meaning

This test clarifies lethal and sub-lethal chemical effects on fish during different growth stages.

b) Experimental method and results

For freshwater species, use Zebra fish, Fathead minnow, Killifish, Rainbow trout; for seawater fish use the Sharphead minnow. Use 3,5-dichlorophenol as a standard compound. The EC_{50} (3 hours) of this compound is 5 - 30 mg/L. Use adult fish, baby fish and fry as samples. The test is conducted in static or flow-through exposure systems, and expose just fertilised eggs to the target chemical at least until all control fish feed freely. During exposure, keep dissolved oxygen at more than 60 % of saturation without aerating. Observe incubation, survival, behaviour, and appearance abnormality every day, and measure body weight and body length at the end of the test. Determine the NOEC and LOEC for each endpoint, such as death rate, survival rate of embryos, baby fish, and fry, and incubation time, body length and body weight, abnormal behaviour / shape, etc.

IV.4.2.3 Multi species mixture ecological toxicity test

Most of the OECD's ecotoxicology tests observe adverse effects on specified single species, except for the test using activated sludge, and the principal viewpoint is distinction of reproduction and other effects to the organisms. It is, however, important to evaluate effects on ecological structure or function in order to evaluate chemical effects on ecosystems. One cannot neglect to understand effects on ecologically important parameters such as energy flow, reciprocal relation, material circulation, change of respiration, self fixation ability, or bioconcentration. Investigation of ecological effects use microcosms or mesocosms consisting of a diverse mixture of species including ecological producers, predators, and decomposers.

A microcosm is a small, model hydrosphere ecosystem, and individuals or groups of organisms are raised in a container environment where it is possible to control nutrition, day-night cycles, temperature, light intensity etc. Several test systems of different size and group composition have been used for ecological effect assessment. There are scene-set-up type, pilot plant type, and flask type systems in which the composition of species is known exactly, and in which it is possible to measure the numbers of individuals, and analyse the character of each species. The system can be maintained under specific conditions and develop after natural selection for one particular group of organisms.

A mesocosm is an isolated field experimental system, isolated from the rest of the hydrospheric environment. There are static types e.g. small lakes and ponds, and flow through systems, such as streams and small rivers.

These chemical ecotoxicology tests using diverse species generally determine ecosystem non-effect concentration, fixable concentration, species co-existence possibility concentration, and ecosystem destruction concentration etc., by observing changes in numbers of individual and groups, ATP (adenosine triphosphatase) activity, and respiration activity etc. However, multi species mixture tests need multi-variate analyses to investigate and assess effects and it is often difficult to report the results as one numeric value, compared to single species tests the results of which are easy to quantify. Therefore, there are many problems in the use of the results of mesocosm and microcosm tests, such as the development of appropriate analytical evaluation methods and clarification of methods for mathematical analyses, and ecological effect tests using mixtures of diverse species are still under research, and haven't yet reached the stage where they are able to be used for regular evaluation of chemical ecological risk.

IV.4.2.4 Cultured cell toxicity test

Instead of *in vivo* toxicity test using individual organism, investigations using *in vitro* test with cultured cell systems have become popular. Test using cultured cells can give results easily and quickly, and it is expected to become a useful method for toxicity assessment by finding correlations with the results of *in vivo* tests.

Fish cells originating from Rainbow trout, Bluegill, Carp, and Killifish etc. can be used. Chemical toxicity in cultured cells are evaluated by measurement and observation of life and death of cells and cell numbers, amount of protein, colony formation, reproduction rate using amounts of DNA, RNA as an index, enzyme activity, form change, DNA damage, chromosome abnormality, and mutagenesis etc.

IV.4.3 QSAR

It is not true to say that ecotoxicology tests have been conducted on all currently used and produced chemicals, nor that toxicity information for risk assessment is available for all such chemicals. Therefore, because of the experience that "chemicals which have similar structure or similar physiochemical characters have similar biological activity, such as toxicity", quantitative structure activity relationships (QSAR) have been developed, and used for prediction of toxicity where data is not available for ecological risk assessment and the confirmation of results of toxicity tests.

In order to estimate toxicity from chemical structures, a value of toxicity to organisms for at least one chemical, chemical structure and reactivity parameters, and statistical tests are needed. Chemical toxicity is caused by a chemical reaction with a target organ after being transferred into the general area of the target organ in the body, so QSAR is expressed by the following empirical formula :

$$\log (1/C) = \text{transport effect} + \text{electron effect} + \text{steric effect} + \text{constant}$$

where C is the molar concentration of chemical causing toxicity. For chemicals which have a fair degree of similarity in their core and active structural components transport, electron, and steric effects are often expressed as substituent parameters. Famous ones are hydrophilicity (π), Hammett constant (σ), and Traft steric substituent constant (Es), respectively. π is expressed as the octanol-water partition coefficient of a substituent, σ is benzoic acid ester, Es is a substituent constant which is determined from hydrolysis reaction rate constant of the aliphatic ester. In order to apply this empirical formula to chemicals which don't have structural similarity, establishment of parameters which can explain the behaviour of not just substituents, but the whole molecule is required. For example, octanol-water partition coefficients, parachor, water solubility, molecular volume, molecular surface area etc. for the transport term; dissociation constant (pKa), molecular orbit index, dipole moment, electron polarity etc. for the electron term; and molecular weight, van der Waals force, molecular volume, molecular shape, molecular surface area, Traft steric substituent constant (Es), molecular refractive index, topological parameter etc..

There are cases where the assumption of similarity is difficult because of the diversity of chemicals to be estimated. Therefore, a QSAR which ignores the similarity assumption is conducted. In general, the accuracy of estimation becomes poor, but practicality increases. That is the method which determines regression formulae for toxicity values and parameters, and each

value and devices of parameter relate to accuracy.

For example, the following relationships between fish acute toxicity (LC_{50}) and MCI (molecular connectivity index, $^3\chi_p$) or octanol-water partition coefficient ($\log P_{ow}$) :

i) molecular connectivity index

$\log (1/LC_{50}) = 3.581 + 0.539\ ^3\chi_p$ (r = 0.769, n = 581)

$\log (1/LC_{50}) = 30.142 + 0.419\ ^3\chi_P - 2.636$ (IP/atom) (r = 0.838, n = 581)

ii) $\log P_{ow}$

$\log (1/LC_{50}) = -3.732 + 0.7351 \log P_{ow}$ (r = 0.783, n = 571)

$\log (1/LC_{50}) = -4.027 + 0.4581 \log P_{ow} + 0.316\ ^3\chi_P$ (r = 0.854, n=571)

where unit of LC_{50} is μmol/L, IP/atom is ionisation potential and determined from the following formula :

IP/atom = (fist ionisation potential of each atom, eV) / Σ atomic numbers

The above regression formulae are obtained without limiting the species of fish, experimental period, and kinds of chemical, and if only compounds which are the same structure series are used, regression formulae with a higher correlation can be obtained.

The OECD puts QSAR application range together as follows : QSAR can only apply to a compound which has normal toxicity, such as anaesthetic compound, and which depends on chemical polarity (e.g. $\log P_{ow}$), liquid compounds at room temperature and a solid compound whose water solubility data is known. The OECD classified chemicals into two categories shown in **Table IV-4-2**. Class I compounds can be used for fish, daphnia, and algae, Class II compounds can be estimated by QSAR of fish acute toxicity.

Table IV-4-2 Categorisation of chemicals for QSAR

classification	structure	applicable QSAR	reliability
Class I	aliphatic alcohols, aliphatic ketones, aliphatic ethers, alkoxy ethers, halogenated aliphatic hydrocarbon, saturated alkanes, halogenated benzenes (containing C, H, N, O, G, Cl)	fish and daphnia acute, chronic toxicity, algal chronic toxicity (non-polar anaesthetic compound)	concentration can be predicted
Class II	non or weak acidic phenol, aromatic amine, aniline, aliphatic primary amine, weak basic pyridine	fish acute toxicity (phenol and aromatic primary amine)	range can be predicted

The following regression formulae can be used:

i) class I

- Fathead minnow (*Pimephales promelas*) 96 hours 50 % lethal concentration (96h-LC_{50})
  $\log LC_{50}$ (mmol/L) = -0.94 $\log P_{ow}$ + 0.94 $\log (0.000068 P_{ow} + 1)$ + 1.75 (r^2 = 0.98, n=65)
- Guppy (*Poecilica reticulata*) 7 and 14 days 50 % lethal concentration (7,14d-LC_{50})
  $\log LC_{50}$ (mmol/L) = -0.87 $\log P_{ow}$ + 1.87 (r^2 = 0.98, n = 60, S = 0.24)
- Fathead minnow (*Pimephales promelas*) and Zebra fish (*Brachydanio rerio*) 28 days no effect concentration (28d-NOEC) and no effect concentration of early life stage test (ELS)
  $\log$ NOEC (mmol/L) = -0.90 $\log P_{ow}$ + 0.8 (r^2 = 0.91, n = 30, S = 0.33)
- Water flea (*Daphnia magna*) 48 hours 50 % free swimming inhibition concentration (48h-EC50)
  $\log EC_{50}$ (mmol/L) = -0.91 $\log P_{ow}$ + 1.72 (r^2 = 0.98, n = 19, S = 0.33)
- Water flea (*Daphnia magna*) 18 - 21 days reproduction inhibition no effect concentration (18-48h-NOEC)
  $\log EC_{50}$ (mmol/L) = -1.04 $\log P_{ow}$ + 1.30 (r^2 = 0.98, n = 17, S = 0.25)
- Water flea (*Daphnia magna*) 18 - 21 days growth inhibition no effect concentration (18-48h-NOEC)
  $\log EC_{50}$ (mmol/L) = -1.07 $\log P_{ow}$ + 1.25 (r^2 = 0.97, n = 10, S = 0.40)
- algae (*Selenastrium capricornutum*) 72 - 96 hours 50 % growth inhibition concentration (72-96h-EC_{50})
  $\log EC_{50}$ (mmol/L) = -1.00 $\log P_{ow}$ + 1.77 (r^2 = 0.93, n = 10, S = 0.17)

ii) Class II

- Fathead minnow (*Pimephales promelas*) 96 hours 50 % lethal concentration (96h-LC_{50})
  $\log LC_{50}$ (mmol/L) = -0.65 $\log P_{ow}$ + 0.7 (r^2 = 0.95, n = 40) phenols, anilines (polar anaesthetic action)
- Fathead minnow (*Pimephales promelas*) 96 hours 50 % lethal concentration (96h-LC_{50})
  $\log LC_{50}$ (mmol/L) = -0.60 $\log P_{ow}$ + 0.6 (r^2 = 0.97, n = 21) phenols (polar anaesthetic action of non-coupling agent)
- Fathead minnow (*Pimephales promelas*) 96 hours 50 % lethal concentration (96h-LC_{50})
  $\log LC_{50}$ (mmol/L) = -0.59 $\log P_{ow}$ + 0.2 (r^2 = 0.98, n = 6) phenols (non-coupling action of oxidising phosphorisation?)
- Fathead minnow (*Pimephales promelas*) 96 hours 50 % lethal concentration (96h-LC_{50})

$\log LC_{50}$ (mmol/L) = -0.67 $\log P_{ow}$ + 0.05 (r^2 = 0.91, n = 11) phenols, anilines (non-coupling agent)

IV.4.4 Use of toxicity data

IV.4.4.1 Prediction of no effect concentration

Methods which predict the no effect concentrations of chemical are complex and not easily understood, and still under research. Thus it is necessary to establish evaluation systems as open systems which always reflect research results and adopt temporary evaluation methods.

Data added with application of chemical registration are generally results of acute toxicity tests. However, ecological risk assessment requires predicted no effect concentrations (PNEC). The PNEC is the concentration which cannot predict some intolerable harmful effects on an ecosystem, and the target actually analysed is a conceptual calculated value. The HPVC project of the OECD expresses it as a maximum tolerable capacity (MTC), but generally the term PNEC is used for the hydrosphere environment,.

For example, ecotoxicology tests using fish include 96h fish acute toxicity tests, prolonged toxicity tests (14 - 21 days), and early life stage tests. However, toxicity data obtained from these tests have different meanings. Therefore, for PNEC prediction it is necessary to put them together in a standardised manner which absorbs the differences in the toxicity data. In addition, predicted PNEC also considers other variables, such as difference between individuals within the same species, species differences, combination of experimental methods, unknown factors etc. Thus factors used for simplification of prediction are called assessment factors (Afs), which have the same meaning as uncertainty factors and safety factors. There is no firm scientific ground to calculate Afs, and countries or international organisations decide them individually.

At the process of refined stage as shown in **Figure IV-4-1**, the insertion method considering different sensitivity between species can be used for PNEC prediction. The insertion method relies on methods of statistical probability, and three methods based on computer programs can be used. In practice, chemicals for which satisfactory toxicity data is available are extremely limited and such application cannot be used in a lot of cases.

By combining the test results using complex systems such as laboratory system using diverse species, microcosm, experimental ponds, field experiments etc., a comprehensive evaluation can be considered. At present, there is no internationally agreed protocol concerning ecosystem experiments, but the US EPA has been developing multispecies test methods, and guidelines have be made by the Society of Environmental Toxicology and Chemistry (SETAC). The OECD recommends criteria in order to judge the usefulness of the results from PNEC prediction by

comprehensive evaluation.

IV.4.4.2 Assessment factors

The OECD's early stage assessment of ecological risk of HPVC which have SIDS against aquatic organisms applies the assessment factors in **Table IV-4-3**, where the environmental concern level (ECL) is the chemical concentrations which can badly affect an ecosystem, and is equivalent to the PNEC and MTC at an early stage. Recommended assessment factors are 10 for the prediction of sensitivity between species, 10 for the prediction of NOEC from acute toxicity, and 10 for the prediction of field conditions from the NOEC giving the lowest chronic toxicity. However, assessment factors are empirical, and there are no scientific grounds for these numbers.

Ecotoxicology test data for aquatic organisms are available from SIDS as basic toxicity data, such as fish acute toxicity, daphnia acute swimming inhibition, and algal growth inhibition (normally acute), and for compounds where there is concern about long term effects, daphnia chronic test data is also available.

If only acute toxicity data is available, factors of 100 - 1,000 are applied to the lowest LC_{50} or EC_{50}. A factor of 1,000 applies as ECL = LC_{50} / (10 x 10 x 10) if only a fish toxicity LC_{50} is available of the three basic acute toxicity tests. This value errs on the side of safety and environmental protection.

Table IV-4-3 OECD's assessment factors for prediction of environmental concern level (ECL)

A. elements of assessment factors to predict ECL from toxicity data of aquatic organisms	assessment factor
1. general application (information applied at the lowest values)	
a. values predicted from chronic toxicity values, NOEC or QSAR from data sets including algae, Crustacea, and fish	10[a)]
b. values predicted from acute toxicity values (LC_{50}, EC_{50}) or QSAR from data sets including algae, Crustacea, and fish at least	100[b)]
c. values predicted from acute toxicity values (LC_{50}, EC_{50}) or QSAR	1,000
2. practical assessment factors from screening information data set (SIDS)	
a. daphnia NOEC, algal NOEC	10[a)]
b. algal EC_{50}, daphnia EC_{50}, fish LC_{50}	100[b)]
B. setting up conditions of assessment factors in order to predict ELC for groups of birds and mammals which live on fish from data of birds and mammals (information applied at the lowest values)	assessment factor
a. at least three NOEC	10
b. less than three NOEC	10[a)]
c. at least three LC50	100[b)]
d. less than three LC_{50}	1,000[c)]

A factor of 100 can be used if there is any of the following evidence：

i) toxicity data covering a wide variety of species can be used if the data includes the most sensitive species
ii) if the structural similarity or QSAR, the ratio of acute vs. chronic toxicity is low compared to many other compounds
iii) if the difference in toxicity between species is small, and chemicals react non-specifically, or like anaesthetics.
iv) chemical discharge into the environment is short term or intermittent, and there is no environmental residues.

When chronic toxicity data is available in addition to acute toxicity data, assessment factors of 10 - 100 are applied to the lowest NOEC after considering the following :

i) If the chronic NOEC of one or two species (fish, daphnia, or algae) which represent one or two nutrition stages, an assessment factor of 50 or 100 may be applied to the lowest NOEC. Compare this PNEC to the PNEC calculated from the lowest acute toxicity data, and make the lowest value the PNEC.
ii) If the chronic NOEC of three species (fish, daphnia, and algae) which represent three nutrition stages are available, a factor of 10 may be applied to the lowest NOEC. If there is confidence that the test was conducted using species which have the highest sensitivity, a factor of 10 can be applied to the lowest NOEC within two species (fish and/or daphnia and/or algae) which represent two nutrition stages. In addition, when assessment factors are changed from basic values, the reason has to be clarified.

A similar method is used to predict safety levels for groups of birds and mammals which live on fish from ecological toxicity data using bird and mammals. In addition, effects on benthic organisms may be assessed. However, in the practical terms they cannot be assessed in many cases because available toxicity data is limited.

Table IV-4-4 compares recommended assessment factors used in the OECD, European Union (EU), and European Chemical Industry, Ecology and Toxicology Centre (ECETOC). The OECD predicts by 10 for every estimation step. Assessment factors used by the EU are different depending on chemical character and test conditions. In general, a factor of 100 can be used between the NOEC and $E(L)C_{50}$ for acute toxicity and chronic toxicity tests. The recommendations of the ECETOC rely on comparisons of toxicity data. It is suggested that ratio of acute toxicity and chronic is 40, the ratio of chronic and ecosystem toxicities, 5, and ecosystem and field, 1.

The OECD says that application of assessment factors is not suitable for metallic forms of

elements and slightly water soluble compounds. Care must be taken when using assessment factors with chemicals which have peculiar properties, such as over 3 log P_{ow}, and show high bioconcentration, and particular attention has to be paid to whether tests were conducted at concentrations which exceeded solubility, or whether test terms were long enough.

Table IV-4-4 Assessment factors for applying toxicity data to aquatic organisms to predict PNEC

information which can be applied	assessment factors which are applied to minimum values		
	OECD	EU	ECETOC
one acute toxicity $L(E)C_{50}$ from one species nutrition stage	1,000	-	-
at least one acute toxicity $L(E)C_{50}$ from each of three species nutrition stage	100	1,000	200
one chronic toxicity NOEC (fish or daphnia)	-	100	-
two chronic toxicity NOEC (fish and/or daphnia and/or algae) from species which represent two nutrition stages	-	50	5
chronic toxicity NOEC from three species (usually fish, daphnia, and algae) which represent three nutrition stages	10	10	5
field observation or diverse species mixture test (model ecosystem)	-	depends on situation	1

note) Algae is not used alone.

IV.4.5 Estimation and judgement of ecological risk

The most convenient and practical method to estimate ecological risk is the quotient method. This method simply divides the environmental concentration or the predicted environmental concentration (PEC) by the toxicological benchmark concentration (TBC). If the value is 1, it can be said that a chemical has the possibility to cause toxic effects. In general, the PNEC or MTC which have been adjusted by assessment factors are used as the TBC. In addition, the NOEC, LC_{50}, and EC_{50} can be used as the TBC.

Initial assessment by the HPVC project of the OECD is undertaken not to judge ecological risk of chemicals directly, but to decide if supplementary testing is necessary. The OECD uses the quotient method for this judgement. That is, if PEC / PNEC < 1, supplementary testing is not a high priority at this point, but if PEC / PNEC = 1, conduct test covering more than the SIDS items or an exposure analysis to assess the risk in more detail. For example, if an estimated PNEC is obtained from only acute toxicity and assessment factors, one strategy would be to conduct a chronic toxicity test using species which showed the highest sensitivity in the acute toxicity test. Also, if there is an indirect effect on birds and mammals, or hazard to benthic organisms living, these assessments have to be considered at the next stage.

Trials to assess the level of ecological effect assumed to be caused by chemicals in the

environment use the ecotoxicological risk quotient (ERQ) as an index. This index shows the level of chemical effect on ecosystem, and is the negative logarithm of the ratio of the concentration in the environment and toxicity standard concentration:

Ecotoxicological risk quotient (ERQ^C)
= -log (environmental concentration / toxicity standard concentration)

Also, an index which shows comprehensive ecotoxicological risk quotient has been proposed assuming there are various chemicals in the environment and they cause effects in a complex manner.

Comprehensive eco-toxicological risk quotient (ERQ^a)
= -log [∑(environmental concentration / toxicity standard concentration)]

The comprehensive ecotoxicological risk quotient is determined assuming that the complex effects caused by the chemicals are neither multiplicative nor suppressive but additive effects in order to simplify quantification This index can be used for regional comparison of the level of ecological risk.

The concentration of a chemical in the environment (its environmental concentration) is the object of risk assessment, and actual measurement data (or predicted values) of concentration in water for water environment, and concentration in soil for soil environment may be used. The LC_{50}, EC_{50}, NOEC, and PNEC and MTCs are being considered as assessment factors obtained from the tests using toxicity standard concentrations.

Because the ERQ differs depending on toxicity standard concentration, the species, test methods and endpoint must be specified, e.g. daphnia ERQ (14d-NOEC), red killifish ERQ (96h-LC_{50}). Also, if the PNEC and MTC being considered as assessment factors are used as standard, the values is described as ERQ (PNEC) because it absorbs species differences in the test and endpoint, and this index can be used for the comprehensive assessment. If the ERQ (PNEC) is more than 0, it indicates that no toxic effects are likely to be caused on organisms in the environment.

Recently, quotient methods like this are the only assessment methods which are often used. The advantage that this method has is that it can be used easily and quickly, and it is easy to understand because there are no complicated mathematics or statistical techniques. Also, the quotient method can multiply multiple chemical risk. The toxicity of chemical mixture is sometimes larger than the sum of the individual chemical toxicities (multiplicative effect), and sometimes smaller than the sum of the individual chemical toxicities (suppressive effect), but it is

assumed that addition of quotient method doesn't have such effects. This assumption doesn't become problem if mode of action of each constituent is the same in the mixture.

It is known that the effects of chemical mixtures whose constituents' mode of action are different are strictly additive for fish acute toxicity test using industrial chemicals, although usually slightly smaller than strict addition, and it is rare to have multiplicative and suppressive effects. However, caution must be used when each constituent in the mixture reacts independently. Also, results obtained from the observation of aquatic organisms may not be applicable to other endpoint, exposure realities, and species. When the mode of action of each chemical is not known, theoretical assumptions about chemical interactions have to be clarified.

Application of quotient method also has many restrictions, because although the quotient method is useful to judge the size of risk, it cannot conduct quantitative assessments, such as the level of effect and possibility of appearance of effects, nor distinguish kinds of effects. For example it is meaningless to predict that a risk reduction policy reduces a quotient value from 25 to 12. This is because one cannot explain clearly the effect on the endpoint caused by quotient value reduction. Also because quotient values do not reflect the intensity of effects or exposure patterns appropriately, LC_{50} values, for example, which are obtained form a continuous series of constant concentration 96 hours exposure test may not be appropriate for reproductive toxicity assessment caused by short or intermittent exposure. Furthermore, the quotient method cannot be used for assessment of secondary effects. This method cannot judge effects of interactions nor effects which exceed the range which can be measured by a simple quotient, such as bioconcentration, because effects from exposure to effect factors spread widely. In order to make good these defects, mathematical model such as the statistical exposure model must be relied upon.

IV.5 Information sources and searches useful for the evaluation of research results

As has been mentioned several times, various bits of information such as chemical substances' names, structures, physiochemical characters, production, use and existence reality, toxicity, standard levels, regulations and the ground etc., are needed during the many stages of planning, data analysis, and risk assessment etc., when environmental monitoring of chemical substances is conducted, and it is an absolute requirement to collect, arrange, analyse and utilise these information. In this section, lists of information sources and data bases which are useful for collection such information are presented. International government organisations and non-government organisations evaluate the safety of chemical substances to meet specific

requirements and officially announce the results for evaluation material. From internet sites many related pieces of information can be obtained.

IV.5.1 Information about compounds

Names of chemical substances are big issues when doing searches because names used by different databases are not always the same. In such cases, Chemical Abstract registry numbers are a useful starting point, and names, alternative names, structures, uses, physical characters, toxicity etc. can be confirmed in a number of ways from books, CD-ROMs, and on-line services e.g.

- RTECS (Registry of Toxic Effects of Chemical Substances) : NIOSH (National Institute for Occupational Safety and Health)
- CA (Chemical Abstract) and CAS On-line : CAS (Chemical Abstracts Service)
- Merck Index
- Sigma-Aldrich (the Sigma-Aldrich Library of Chemical Safety Data) : Sigma Aldrich Corp.

IV.5.2 Information about toxicity

Information about toxicity, adverse effects and methods of evaluation can be obtained from the following.

- RTECS (Registry of Toxic Effects of Chemical Substances)
- HSDB (Hazardous Substances Data Bank: NLM (CD ROM)
- IARC Monographs on the Evaluation of Carcinogenic Risks to Humans : IARC (International Agency for Research on Cancer)
- EMBASE: STN International/DIALOG (On-line Network Service)
- Pharm-Web (Internet, http://www.pharmweb.net/)

IV.5.3 General information

General information which is useful for risk assessment may be obtained from the following sources.

- WHO Technical Report Series: WHO (World Health Organization of United Nations).
  Activity report of WHO.

- Environmental Health Criteria : IPCS (International Programme on Chemical Safety)

  General evaluation of the effects on humans and the environment of pesticides, chemical industrial products, environmental pollutants, natural poisons, etc.
- Health and Safety Guide : IPCS

  Guides to the management and treatment of accidental exposure to chemical substances such as pesticides, chemical industrial products, environmental pollutants, natural poisons, etc.
- IARC Monographs

  General information useful for risk assessment centering around carcinogenic risk evaluation for humans to such things as natural materials, pharmaceutical products, chemical industrial products, pesticides, environmental pollutants etc.
- IARC Scientific Publication

  Scientific reports on carcinogenic risk (research about methodology)
- Pesticide Residues in Food, Evaluation, Report JMPR (FAO/WHO Joint Meeting on Pesticide Residues, Food and Agriculture Organization of United Nations)

  Recommendations of ADI and MRL (Maximum Residue Limit) of Pesticides.
- Joint Assessment of Commodity Chemicals : ECETOC (European Chemical Industry Ecology and Technology Centre)

  Evaluation of the effects on humans and the environment of currently existing industrial chemical products.
- Technical Report : ECETOC

  Review of scientific articles about potentially harmful industrial chemical products.
- Monograph : ECETOC

  Discussion about evaluation methods of toxicity.
- Technical Report : NTP (National Toxicology Program)

  Evaluation by toxicity tests of the carcinogenicity of chemicals to which humans are exposed and for which research value is high.
- Toxicological Profile : ATSDR (Agency for Toxic Substances and Disease Registry)

  Evaluation of the health effects of harmful wastes, such as chemicals which are buried in large quantity as harmful wastes.
- Federal Register

  US official gazette. Analytical methods, instruction of evaluation, regulation etc. are listed in detail.
- Concise International Chemical Assessment Document

  Summarising data on risk assessment of chemicals by international co-operation,

exposure indexes are introduced.

- International Chemical Safety Card

  Summarises acute effects on humans and the physical danger of chemicals.
- Toxicity of Chemicals, Carcinogenicity : EC (European Communities)
- OECD (Organization for Economic Cooperation and Development) SIDS Initial Assessment Report

IV.5.4 Internet sites or contact address

IV.5.4.1 Information of safety evaluation and data base

- Chem Finder : http://chemfinder.camsoft.com/
  (chemical structures, physical characters etc.)
- Concise International Chemical Assessment Document :
  http://www.who.ch/programmes/WHOProgrammes.html
- ECDIN : http://ulisse.etoit.eudra.org/Ecdrin/Ecdrin.html
  (environmental chemicals data base of EU)
- Environmental Health Criteria : gopher://gopher.who.ch:70/11/.pcs/.ehc
  (summary of recent EHC)
- International Chemical Safety Card : http://www.nihs.go.jp/ICSC/
- International Uniform Chemical Information Database (IUCLID): European Union
  (EU data base of currently existing compounds)
- KIS-NET : http://www.fsinet.or.jp/~k-center/k-p5.htm
  (chemical safety information system of Kanagawa prefecture)
- National Toxicology Program: http:///ntp-server.niehs.nih.gov
  (test information, publication, safety information, chemical structures information, etc. of US NTP)
- NORDBAS : Nordic Council of Ministries (Environmental Hazard
  Classification-classification of selected substances as dangerous for environment)
- SWEDEN KEMI : http://db.nihs.go.jp/dcbi/genera/KEM/
  (compounds list of KEMI Sunset Project)
- Toxicological Profile Query : http://atsdrl.atsdr.cdc.gov:8080/gsql/toxprof.script
  (Summary of Toxicological Profile of US ATSDR)
- US Federal Register : http://cos.gdb.org/repos/fr/fr-intro.html
  (US official gazette)

IV.5.4.2 International organisations

The following organisations web sites can accessed for information.

- CIS/ILO [International Labor Office (ILO) International Occupational Safety and Health Information Centre (CIS)] : http//turva.me.tut.fi/cis/home.html
- EC (European Communities) : Office for Official Publications of the European Communities, 2, Rue Mercier L-2985 Luxembourg (reference of Toxicity of Chemicals, Carcinogenicity)
- ECETOC : http://www.nihs.go.jp/guide/ingovel.html#ECETOC (reference of Technical Report, Joint Assessment of Commodity Chemicals)
- IARC : http://www.iarc.fr/ (link to information of IARC)
- OECD's Work on Environmental Health and Safety : http://www.oecd.org/ehs (OECD information of chemicals, prevention of accidents, pesticides plan etc.)
- WHO: http://www.who.ch/ (link to WHO information)

IV.5.4.3 Governments of Europe, US, and Australia, and related organisations

- Australia : http://www.erin.gov.au/portfolio/epg/epg.html (Australian Priority Existing Chemicals Program)
- Environment Canada Health Canada : Ministry of Supply and Services, Canada Communication Group, Ottawa, KIA0S9, Canada (Canada Priority Substances List Assessment Report)
- BUA GDCh-Advisary Committee on Existing Chemicals of Environmental Relevance : Gesellschaft Deutscher Chemiker E.V. BUA, Postfach 10 14 80 D-60444 Frankfurt, Germany (BUA Report)
- BG Chemie : Berufsgenossenschaft der Chmeischen Industrie, Postfach 10 14 80 D-6900, Heiderberg 1, Germany (Toxicological Evaluation)
- MAK Commission : VCH Verlagsgeselshaft, Postfach 10 11 61, D-6940 Weinheim, Germany (MAK Evaluations)
- Health Council of Netherlands (GR) : Gezondheidsraad Postbus 90517 NL-2509LM's Gravenhag, Netherlands (Basis-Document, Criteria-Document)
- RIVM : Rijksinstituut voor Volksgezondheid en Mimieuhygiene (RIVM), Postbus 1 NL-3720 BA Bilthoven, Netherlands (Integrated Criteria Document)

- Dutch Expert Committee on Occupational Standards (WGD) : WGD Werkgroep van Deskundigen (WGD), Gezondheidsraad, secretariaat WGD Postbus 90517 NL-2509 LM DenHaag, Netherlands, (Health-based Recommended Exposure Limit)
- Swedish National Chemicals Inspectorate : http://db.nihs.go.jp/genera/KEMI/contents.html (KEMI Report Series)
- BIBRA Toxicology International : BIBRA Information and Advisory Service, Woodmansterm Rd, Carshalton Surrey SM5 4DS, UK (Toxicity Profiles)
- Health and Safety Executive (HSE) : HSE Books. P.O. Box 1999 Sudbury, Suffolk CO10 6FS, UK (Criteria Document for an Occupational Exposure Limit)
- US EPA : http://www.epa.gov/ (link to all information of EPA such as HAD : Health Assessment Document, Hazard Information Profiles, IRIS : Integrated Risk Information System)
- US NIOSH (National Institute for Occupational Safety and Health) : http://www.cdc.gov/niosh/homepage (publication, explanation of data base of NIOSH)
- US FDA(Food and Drug Administration) : http://www.fda.gov/fdahomepage.html
- National Institute for Occupational Safety and Health (NIOSH): http://www.cdc.gov/niosh/homepage (Criteria Documents)
- National Toxicology Program (NTP) : http://ntp-server.niehs.nih.gov (NTP Technical Report, NTP Toxicity Report)
- American Conference of Governmental Industrial Hygienists (ACGIH) : http://www.acgih.org/ (Threshold Limit Values and Biological Exposure Indices)
- Agency for Toxic Substances an disease Registry (ATSDR) : http://atsdrl.atsdr.cdc.gov:8080/gsql/toxprof.script (Toxicological Profile)
- CIR Panel of Experts : Cosmetic Ingredients Review, 1110 Vermont Ave., N.W., Suite 810, Washington DC, 20005, USA (CIR : Cosmetic Ingredient Review)

IV.5.4.4 Japanese government and related organisations

- Environment Agency of Japan : http://www.eic.or.jp/
- Ministry of Health and Welfare : http://www.mhw.go.jp/
- National Institute for Environmental Studies : http://www.nies.go.jp/
- National Institute of Health Sciences : http://www.nihs.go.jp/
- National Institute for Resources and Environment : http://www.nire.go.jp/

V Quality control : guidelines for achieving quality in trace analysis

V.1 Introduction

This chapter explains those matters in trace chemical analysis to which special attention should be paid in order to obtain reliable results (such as the analysis of inorganic and organic compounds at concentrations less than 100 ppm). This chapter describes general methods for both quality control and techniques for trace analysis. For example, requirements for the 'ideal laboratory', and concerns about apparatus, analytical methods, and routine analysis. Specific analytical methods are not discussed, and the nature and size of errors and the technology required to decrease such errors are not mentioned. One way to use this chapter is as a concise outline or as technical notes which should be followed in the laboratory.

As analytical technology improves, so instrument sensitivities are improved and the amount which can be detected becomes smaller. One consequence of this instrumental improvement is that, unless analysts pay attention to such matters as contamination or leakage at the time of trace measurement, the accuracy of data becomes unreliable and errors in the data can be large. It is absolutely necessary to make strenuous efforts to maintain low and constant blank values, and important to prevent contamination. Advanced analytical instruments and contamination management can make the evaluation of lower concentration samples possible. Needless to say, evaluation of the results of such trace analysis must also be paid sufficient attention.

V.2 Apparatus and instruments

Ideally, all apparatus and instruments used for trace analysis should be located and maintained separately from equipment used for general analysis. If that is not possible, some management strategies to prevent the risk of contamination when apparatus and instruments are shared, have to be adopted.

Trace analysis is very different from macroanalysis. Essentially, this is because small amounts of contamination in the final sample solution (the solution used for determination) causes the bulk

of the error. Such contamination can be derived not only from the apparatus or reagents but also from such things as building materials, the environment, or analytical operations and procedures, etc. To prevent contamination the area or areas used for trace analysis should preferably be completely separated from those used for macroanalysis. If such areas are not available, one must use contamination free cabinets or a part of the laboratory for trace analysis.

Apparatus used for trace analysis should be exclusive to such trace analysis studies. One must take special care not to share laboratory, solvent, glassware, or other apparatus to avoid contamination problems. If it is possible, use disposable glassware or plastic apparatus. Also, one must consider what other operations are being conducted in the same area. Wash apparatus or equipment severely, and contamination must be minimal for blank samples. Do not bring samples which contain high concentrations of target compounds into the trace analysis area.

V.2.1 Laboratory

The laboratory and associated equipment should be designed to allow analysts to work easily. The materials from which the laboratory is built may affect the analytical results. Building materials should be resistant to corrosion caused by the chemicals being used.

a) The working environment should be appropriate for trace analysis. There should be minimal possibility of contamination of samples. Activities such as eating, drinking, smoking, and applying make-up should all be banned in the laboratory.

Sometimes special laboratories built as clean rooms are necessary. When clean rooms are not available because of lack of budget or other restrictions, a minimally contaminated area or clean cabinet should be used. Consider what the other work is being undertaken in the same room or next door laboratory. If there are gases or particles in the atmosphere, it is possible to decrease their potential contamination effect by exchanging air. If the working area is small, it is good idea to cover apparatus and instruments to prevent particle adhesion. One must also consider the different requirements of trace inorganic and organic compounds determination. Cosmetics or jewels should be avoided because they are possible sources of contamination. Hair should be covered. Gloves reduce contamination compared to using bare hands. However, one must be careful which gloves one chooses, because the gloves themselves might pollute samples (rubber gloves contain dithiocarbamate as a vulcanisation agent, and plastic gloves may contain metals).

b) In order to gain reliable analytical results, the temperature in the laboratory should be well controlled, and a record should be maintained.

Indoor temperature may have an effect on the physical measurement conducted in the laboratory. For example, the volume of volumetric glassware is determined at a certain temperature. There are some operations effected by temperature such as extraction with ether, or degradation of target compounds during sample separation. Change of ambient temperature affects the character of HPLC columns and other instruments. When experiments are conducted at specific, non-ambient temperatures, the temperature should be measured and a record maintained.

V.2.2 Analysts

To obtain good results for trace analysis, staff must always consider the details of their work, which aspects should be paid particular attention, what aspects specifically affect results. Good results rely on the experience and cautiousness of the analysts. To obtain consistent results, staff must take pride in their work.

a) The analyst must be carefully chosen from those who are experienced and have both a deep understanding and knowledge of the requirements of trace analysis.

The analyst must thoroughly understand the analysis and related matters, and be able to scientifically answer questions about their analytical results. The analyst must understand well specific analyses, and be able to evaluate the "suitability for purpose" of the adopted method.

b) Other technical staff working under the analyst must also be well trained, analytically skilled, and have experience of dealing with the apparatus, instrument operation, data analysis. Such staff should know the purpose of the experiments, the problems inherent in each level of operational procedure, the necessity of considering special problems specifically related to the samples, the importance of accurate operation, and the size and relevance of the inevitable errors.

Staff must, ideally, understand the basic principles of the analytical operations and the necessity of quality control systems. If any member of staff has little experience in these matters then the whole process should be closely supervised by an experienced person. An inexperienced staff member should not be allowed to report on his own responsibility until he obtains sufficient

analytical skill under the supervision of the experienced person. All staff have to be familiar with the operation of general experimental apparatus such as balances, volumetric apparatus, and know both how to use such equipment and the error generated in their use. Staff must undertake training seminars and programs related to analytical methods and their work.

c) Records concerning the training history (external in-house seminars and training programs) experience and personal character of all analytical staff should be kept.

Laboratories and institutes should plan regular seminars, the subjects of which are based the operational requirements and general experience of staff.

V.2.3 Laboratory infrastructure

a) Laboratories should be provided with stable and guaranteed quality supplies of electricity, gases, and water.

It may seem self-evident, but a stable supply of electricity, water and gas is important to conduct reliable trace analysis. A stable electric supply is necessary to allow many instruments work in a reliable and stable manner. Many laboratory operations require deionised water. many institutes provide a centralised deionised water supply system, but it is often necessary to re-purify such deionised water depending on the nature of the analysis. Purity of gases have to be checked regularly. Gases can be purified by using such filters such as molecular sieves, anhydrous salts, activated charcoal, or deoxygenating traps.

b) It must be stressed that for trace analysis to be conducted in a laboratory, the condition of reagents, solvents, and experimental apparatus should be appropriate for such investigations.

Reagent and solvent blanks must be measured (checked) regularly to confirm that there is no contamination. If blank value show high concentrations of target analytes, check all reagents to find the source of contamination. It is relatively easy to measure reagent contamination using the same analytical method as ones used to measure samples. In addition, purified water must be analysed regularly to determine if the water contains target compounds or other interference. If detergents are used for washing glassware, one must rinse the glassware thoroughly so there is no detergent residue. Depending on the purpose of apparatus, organic solvents, acid, or alkaline may have to be used for the last rinse.

c) Keep records confirming the good condition and maintenance of all apparatus.

These rules apply equally to specific analytical instruments as well as general apparatus in the laboratory. The temperature of refrigerator and freezer s in which samples and standards are stored should be monitored and recorded.

It may be necessary to adjust or reconfigure instruments, including making calibration curves as mentioned in V.4.5. This type of adjustment corrects problems to do with the character of the instruments. For example, there are chemical scale adjustments, wavelength scale adjustments of ultraviolet spectrophotometer, or mass adjustments of mass spectrometer.

An instrumental "Ability Test " may have to be performed under the supervision of the chief analyst in order to check if capability of the instruments is at an appropriate level. Such tests include measurement of single or multiple standard solutions to check sensitivity, resolution, noise, and baseline drifting. The frequency of such tests depends on change in instrument capability. When instruments don't work as they are supposed to, indicate by means of a warning note that the instrument may not be in an appropriate condition for use. And before the instrument is used again, the proper repairs, re-calibration, re-tuning, etc. have to be done. Also even if there are no problems, regular maintenance is necessary. Records of routine maintenance and re-calibration must be kept along with the analytical results.

V.2.4 Analytical standards and reference materials

The term "analytical standards" is widely used by and between analysts. However, the term "analytical standard" used in this chapter means compounds and solutions which are used for making calibration curves or used for instrument checks. The ISO distinguishes this definition from that of "reference materials" as a piece of basic and general terminology required by analysts.

a) In the laboratory, analytical standards whose purity or composition is known have to be used.

Use commercial standards which come with appropriate purity notes and quality control warranties. Purchase the best standards possible. Certified reference materials designated for use in the preparation of calibration curves are recommended if they are available. If it is not

possible to purchase standards whose concentration or compositions are known, prepare your own analytical standards, then determine the concentrations according to designated methods.

b) Standards have to checked for reliability and certainty before use in trace analysis.

Check to see if the material is of the designated grade reagent. An easy way to confirm this is to compare newly obtained standards to old ones. If there is difference, it will be necessary to investigate the discrepancy further.

c) When standards are used, the concentration of the standards, and how accurately the concentration has been determined, should be known. It is necessary to examine thoroughly whether the concentrations and the accuracy of their determination are sufficient for the purpose of the analysis.

The concentration of analytical standard solutions has to be known with sufficient accuracy to make sure it wouldn't cause analytical errors. In order to evaluate the inaccuracy of the whole analytical procedure, the accuracy of the standards has to be known. Control of standard stock solutions can be achieved by limiting use, keeping an appropriate reserve, and recording use. Make a standards log book to keep records. Standard stock solutions and the diluted standard solutions should be prepared by a designated person. Records of measured weights of standards, volumes of flasks and pipettes used for the preparation should be kept with signature of the person who prepared the standards.

d) Analytical standards and standard stock solutions have to stored under such conditions that will not cause their concentration to change. The preparation dates and expiry dates should be clarified on the labels on the bottles.

Analytical standards should not be stored with samples, or in places which may causes contamination.

Analytical standards and standard stocks deteriorate as time goes by. The expiry date of such solutions depends on the target compounds, their stability in the solvent, the stability of the solvent, and storage conditions. Expiry dates can be obtained from literature data, information from other analysts in the same field, and experiments. Keep detailed records of how the length of storage was determined. It is necessary to describe in the analytical methods about how long and how analytical standards and stock solutions should be stored.

Reagents for making standard solutions and analytical standards also have to stored. In this

case, in sealed containers in the dark at 1 - 5°C. Working standards (low concentration standard solutions in frequent use) should not generally be used for more than 2 - 3 months. Compounds which easily evaporate and are decomposed should of necessity be prepared every time.

e) Traceability of measurement results must be secured using standards with guaranteed values.

Traceability is important to allow comparison of data from different institutes and countries. If analytical methods don't cause systematic errors and standards for calibration curves are assured in nationally or internationally, traceability can be secured. Traceability of physical measurement is easy, but traceability of chemical analysis can be secured by analysing certified reference materials (CRM) which have a similar composition to one's samples and whose composition has been determined nationally or in international institutes. If ideal reference materials cannot be obtained, use the best available substitutes. For example CRM which have similar composition may cause the same problems from the point of analysis as actual samples. It is inevitable that one may have to purchase reference materials which don't contain clear information about traceability. In such cases obtain additional information from the maker. NIST (USA), NRC (Canada), BCR, IAEA (EC), and NIES (Japan) all provide environmental reference materials.

V.3 Methods

V.3.1 Plan

A good plan is indispensable for successful trace analysis. When formulating the plan, consider the purpose of the analysis, analytical quality control, the sampling regime, the choice of analytical methods, the accuracy and precision of the methods, and report contents etc.

Consider comparative analyses to evaluate contamination levels. The analytical uncertainty has to be evaluated and reported as a part of routine work of laboratories.

V.3.2 Selection / development of analytical methods

a) Cautious selection and development of analytical methods is crucial to obtain reliable results.

There are several analytical methods that may be applied to most studies. The following list shows, in order of importance, which methods might be applied for most purposes:

(1) Official analytical methods being simultaneously compared and unified at multiple institutions at a very high technical level.

(2) Analytical methods whose adequacy has been confirmed at more than two institutes, or which are recommended by specialist committees.

(3) Analytical methods have been developed by institute itself and whose adequacy has been confirmed. Original methods from the literature, books or various manuals.

b) Important factors that influence the choice of analytical method are whether sufficiently reliable results can be obtained under such limitations as available experimental apparatus, instruments, required time and time limits, budget and other restrictions.

Analysts can generally obtain accurate results by using common, familiar analytical methods instead of using completely new methods. In order for analysts to be able to apply an adopted an unfamiliar, analytical method to samples with special problems having never personally applied the method, it will be necessary for the analysts to check for themselves both if the method is appropriate, and if the method is as reported. (Ref. V.3.3)

Ideally, one should use the largest quantity of sample possible given the limits of sample size and analytical constraints. This is especially important when target compounds are not evenly distributed in the sample matrix. Large amount of samples contain more target compounds, there are less contamination effects, and decreased operational losses. The most important technical points for choosing analytical methods are:

(1) How much analytical accuracy is necessary? Does the chosen method satisfy this requirement?

(2) Are measurement results within the range of the calibration curve used?

(3) Is the detection limit of the chosen analytical method lower than the expected concentration of the constituent in the sample ?

(4) Are there any interferences in the samples? Was a spike recovery test done using actual samples (spiked amount is equivalent to the amount in the sample)?

(5) Are instruments, reagents, apparatus ready for analysis? Were staff appropriately trained to operate the analytical methods?

(6) According to historical data for the selected analytical method, did results of inter-laboratory analysis or the repeatability reported by each institute agree well? How much difference is there between standard deviations of repeatability test within the same samples and that of one test operated on the whole process?

c) The two most important factors which decrease opportunities for contamination and loss of target compounds are a decrease in the number and complexity of analytical operations. However, interferences which have effects on the measurement results of the target compounds should be removed until the effects the interferences cause can be neglected.

Any reduction in the number of procedures is always related to removal of interference. Increasing the number of procedures increases the possibility of contamination, and decreases accuracy. For example, when target compounds are extracted from samples with solvents, direct measurement of the extracts is highly accurate, compared to extraction of target compounds through additional operations such as reverse extraction and solvent removal.

V.3.3 Adequacy

a) Accuracy and precision have to be checked through the whole analytical process. Precision can be checked by analysing standards. If standards are not available, precision can be confirmed by comparing the data being collected with that from another analytical method whose principles are different and whose reliability has been confirmed.

Accuracy has to be checked not only at the last determination step, but throughout the whole process. Accuracy can be checked by analysing multiple, homogeneous samples which contain multiple target compounds. Precision may also be checked by analysing spiked samples. Inclination (systematic error) can be checked by analysing samples of known composition such as standards or determining recovery of spiked samples.

b) Confirmation and evaluation of the causes of inaccuracy should be undertaken.

It is important to know the difference between inaccuracy and error. Inaccuracy of analysis gives data incompleteness. Analytical error is a value that causes the analytical value to deviate from the true value. Therefore, if the analytical error is known, data can be adjusted to the true

values.

V.3.4 Quality control system

It is necessary to establish internal quality control systems which are clearly regulated, in order to monitor performance of instruments, reliability of calibration curves and dispersion or inclination through the whole analytical procedure. This can be checked by regularly analysing compounds which are close to the composition of the samples.

Systematic internal quality control has to be conducted as a part of normal quality control in order to investigate everyday or batch analytical conditions. Prepare a manual which clearly explains the procedures. The nature of quality control system depend on the importance and character of analysis, the frequency of analysis, batch size, automation capacity, difficulty of the analytical method and reliability. Confirmation of analytical results by quality control should be done for each batch. If the check samples are outside prescribed limits, abandon the results of all samples after the last check samples which give a normal result. Then conduct appropriate improvement before re-analysis of samples. Samples for quality control (QC) have to be typical samples, stable, and in a sufficient amount to be used for long periods. During the study period, whether the analytical methods fits in a prescribed range can be checked by plotting analytical values of QC samples on a normal chart. The amount of QC that has to be conducted depends on the nature of the study, but must be sufficient to prove the reliability of the analytical data. For example, it is normal to analyse one QC sample after every 20 samples. For complicated analyses, analysis of 30 % of samples as QC samples is not uncommon, sometimes more than 50 % is necessary. If the analysis is rarely conducted, analysis character tests have to be undertaken each time the method is used. This includes analysis of standards (reference materials) whose concentration are known, double analysis, and recovery tests. If the analysis is conducted more often, systematic QC using control charts and check samples has to be undertaken.

Essentially, quality control plans have to include the following :

- **Regular checks for contamination.**
- **Regular recovery tests using analyte concentrations similar to that in the samples to evaluate analytical method operation. Use the same matrix as sample for recovery test.**
- **Analysis of check samples for each group of sample.**

V.3.5 Documenting analytical methods

The analytical methods and all routine operating procedures have to be documented. The document should contain information detailing the collection and nature of samples, details of the analytical procedure, detection limits, methods for calculating analyte concentrations. Also it has to be clear who is responsible for the analytical methods and who has authority to change the method.

The document should be stored for a pre-determined period of time, and if some changes and improvements happen, the changes have to be made obvious in the documentation. Written methods are described and sorted as “analytical methods”, “standard analytical methods”, “standard operation procedure”, “business order?”, “protocol” etc.

V.4 Analysis

No part of analytical procedure must change the composition of the samples, or affect the concentration or determination of the target compounds.

V.4.1 Receiving and storage of samples

A reliable system for the registration and record of samples in the laboratory must be established. Samples which are brought in and the requested form of analysis should be compared and checked. Make records of any damage, or abnormality of the sample containers and the samples at the time they are received. Record the dates and the time the samples are received. Open any packages carefully, in a safe place and with the appropriate level of safety precautions, and in a place which has no, or minimal, risk of contamination. Mark the sample with unique numbers (codes) which can be used from the moment of sample receipt, through the analysis to the reporting of the results.

Analyse unstable samples immediately. If this is not possible, or treat and store the sample in a manner which prevents sample decomposition or change. There are several things to remember when storing and preserving samples. Although light affects only certain kind of compounds,

shielding is generally necessary. To prevent evaporation of volatile compounds, it is necessary to pay special attention to temperature, exposure to sunlight, and the integrity of container seals. The stability of samples, standards, and standard solutions is a function of standing time at each step of the analytical procedure. Samples must be stored in appropriate containers under appropriate conditions which prevent cross contamination with other samples, do not allow decomposition by external factors such as light and heat, and preserve the sample. High concentration standards and samples should be stored separately from calibration curve standards because of the possibility of contamination.

V.4.2 Taking sub-samples

Check visually if the samples contain objects which have to be removed. If there is any doubt about homogeneity of samples, mix the sample thoroughly. When not all of the sample is used for analysis, take representative sub-samples. Be careful of contamination and chemical changes of target compounds or sample matrices when separating samples.

When sub-samples are taken from inhomogeneous samples, special care is necessary. It may be possible to determine from exceptional data which component of the samples has to be chosen. However, most of the time samples have to be homogenised evenly.

V.4.3 Sample preparation

Make sure that extraction or dissolution conditions (temperature etc.) do not cause decomposition or decrease of concentration of target compounds in the sample. Reduce interferences and contamination. Pay attention not to spill sample solutions or cause any loss by adsorption and desorption.

V.4.4 Measurement

Describe the operation of analytical instruments clearly, so there is no chance of any misunderstanding. Conduct regular maintenance of instruments at appropriate intervals. Mention anything which may affect instrument sensitivity. One MUST operate the instruments

within the limits of the range of the calibration curve or optimum operating range. Check reproducibility for sample measurement beforehand.

Describe in detail in written analytical methods the making of calibration curves, the frequency of measurement of blanks, standards and check samples. Write down details of the operating range of instruments. Conduct work according to such outlines of operations e.g. for steps such as set up of instruments, judgement of ability, operating conditions and operations.

Describe in detail the possibility of interference and appropriate adjustment methods. Adjustments are done by using solutions which contain known concentrations of both target compounds and other compounds whose concentrations are the same as samples.

V.4.5 Making calibration curves

Measure standards repeatedly at designated intervals to make instrument calibration curves and to adjust results for changes in instrument sensitivity during measurement. Measure reagent blank as necessary in order to check if there is any residual contamination after standard measurement. Measurement of standards is also used to check if the reproducibility of results is within an acceptable range.

It is hard to stress how important it is to make calibration curves properly in order to gain accurate results. There are several kinds of calibration curves, and which one to choose depends on the character of the samples to be analysed and the analytical instruments to be used.

- The absolute calibration curve method (external calibration curve) measures concentrations of standard solutions in an operation distinct from that of sample analysis. A standard solution containing a single concentration of the target analyte is analysed and a concentration-response factor (RF) determined. By using standard solutions of multiple concentrations a calibration curves can be plotted. Once the calibration curve is produced, the response of the target analyte in the samples is used to determine the concentrations of the target compounds in the sample.
- The internal standard method is used to decrease errors for chromatography or atomic absorption spectrometry. There are two types of internal standard method as the following:
  (1) General internal standard method. Calibration curves are produced in a similar manner to the external standard method for compounds of similar chromatographic behaviour to

the target analytes. Thereafter, known amounts of these compounds are added to the sample mixture either prior to any sample preparation or just prior to chromatography. The ratio of the response of the internal standard and the response of the target analyte in the samples is used to determine the concentrations of the target compounds in the sample. A variation of this method is the isotope dissolution method for mass spectrometry.

(2) Standard addition method. Used to determine the change in response caused by the solution being introduced into analytical instruments. Internal standards are added to both standard solutions and samples, the standard and samples are measured together, and the response of the additional target analyte in the sample is used to determine the concentrations of the target compounds in the sample.

Calibration curves should be made regularly in order to confirm the sensitivity and / or drift in sensitivity of analytical instruments. Record all apparatus parameters at the time of taking data for calibration curves because change in apparatus parameters can affect the slope of calibration curves. Calibration conditions can change abruptly depending on continuous parameter change or conditions of analytical operation. In order to cope with this, most laboratories conduct regular re-calibration. Regular calibration is indispensable for formal quality assurance schemes.

V.4.6 Signal management

a) Signal management in analytical methods is very important. The technology involved should not readily be changed without special reasons. If the technology has to be changed, write down the reasons for the change as well as details of the alterations.

Signal management technology is used to change the electrical signals originating from the detectors to forms more meaningful for analysis. Signal management includes signal amplification, alternating current signal rectification, reduction of background signal, exchange from analogue to digital, and integration etc.

b) Data can be used only when the S/N ratio is more than the value specified in the analytical methods.

Accuracy of measurement relies on the S/N ratio at the time of measurement. The permitted S/N ratio is given in the analytical methods, the S/N ratio during the analysis of a series of samples should be above that. Otherwise the data obtained will not be in the range determined by the

methods.

c) It is necessary to pay attention to signals produced by blanks. If such signals are not within tolerance limits, all measurement data at that time are invalid.

If blank values have an adverse affects on analytical results, the blank cannot be accepted. If the response of blank is extremely large, contamination from reagent or solvent may be the reason. The reason of contamination has to be searched because contamination gives analytical results uncertainty.

V.4.7 Confirmation analysis

If more reliability and accuracy than normally obtained by the method of analysis is required, conduct additional confirmatory analysis. This can be done by using different analytical methods or different standards. Confirmation of detected and not-detected concentrations is also important. Both determination and confirmation of concentration of target compounds are necessary. It is sometimes indispensable for trace analysis to confirm both compound identity and concentration by another analytical method.

To confirm is to analyse by more than two analytical methods. Reproducibility can be evaluated by analysing several times by one analytical method. Only reproducibility information can be gained by repeating analysis using the same analytical instruments. Elemental analysis can be confirmed by analysing by two analytical methods based on different physical principles.

When the results are unexpected, extremely big or small , a second and third sub-sample should be analysed by another analytical method to confirm the results.

V.4.8 Dealing with raw data and reports

a) Analysts must follow recognised protocols if data adjustment have been made on the basis of recovery data. A report generally has to accompany the results explaining such matters as how data was calculated and data handling procedures etc. When more than two analytical systems are used to produce results, describe how the two sets results are linked together. When new or complex statistics or mathematical methods are used to calculate the results, it is necessary to explain how and why the methods are appropriate. Data values which are less than detection limits should not be reported.

If data adjustment is made on the basis of recovery data, write down the details of the calculation and make the values before adjustment available.

b) It is necessary to determine detection limits, determination limits and report limits. Laboratory institutes have to evaluate their own detection limits and determination limits using samples.

Institutes have to confirm determination limits by using spiked samples or samples with known concentrations of target compounds. Spiked samples are made by adding target compounds whose concentrations are around expected determination limits into samples which don't contain target compounds.

c) All results have to be reported in a clear format (generally written documents). Reported results have to relate clearly to analysed samples' names. Also report limit values and determination limit values, and an estimation of the uncertainty of results must be described. An outline of the analytical methods used can be inserted in order to aid understanding of the results and the limits of their application. Data which must be reported are blank values, recovery results, and results of repeated examination. All reports have to be checked for mistakes by the person or persons in charge.

The results of calculations using electric calculators and computers are often un-necessarily detailed (too many significant figures) so they have to be rounded up to more appropriate numbers.

When analysis has been repeated, show results with an average ± standard deviation if the number of repeats is large enough. If the number of repeats is small, show results as range. Standard deviation numbers are rounded up to one significant figure, and averages are rounded up to the appropriate number of significant figures balancing accuracy and the standard deviation.

V.4.9 Maintenance of records and storage of data

All data related to the analytical results must stored for a specified period of time to allow investigations of each step of analysis if required afterwards. Data that must stored are analysis request forms, estimates, sample record book, analytical results (data sheets from analytical instruments), calculation processes, record sheet of results, experiment note book, calibration curves, operation procedure, conditions of analytical instruments, etc.

Each record has to be correlated to samples, and reserved with sign of the analyst with dates in order to be able to specify all records about analysis when necessary.

V.5 Monitoring and inspection

a) Using confirmed analytical methods, professional analytical institutes, and veteran analysts does not always guaranteed reliable analytical results. In order to decrease the chance of causing errors, all analytical procedures have to be conducted using a system which guarantees the quality of the analytical results.

Quality assurance in trace analysis requires analysts to maintain a control chart. A control chart is useful for error detection. However it only can correct errors, it cannot prevent errors which arise at the beginning. Quality assurance systems prevent errors. Therefore quality goes up and efficiency increases because of error elimination.

b) At regular intervals, internal and external inspections have to be conducted to guarantee that the quality assurance program is working appropriately.

c) Institutes should participate in technology confirmation tests or inter-laboratory research as a quality control on their analytical results. Such tests make direct comparisons of in-house results with results for the same samples obtained by other institutes, and it is useful for checking technology. If possible, when conducting such tests, add check samples into routine analysis so the analysts can conduct the analysis under the normal, working conditions. Quality control systems should be established.

V.6 Appendix

Some especially important subjects that influence quality control in a chemistry laboratory are :-

V.6.1 Staff

(1) Train staff appropriately and keep accurate and up-to-date training records.
(2) Check the analytical ability of staff.
(3) Tests of analytical ability of staff have to be done by analysts who have both authority and the ability themselves.

V.6.2 Apparatus

(1) Gain certification of apparatus correction.
(2) Stick the label about correction of apparatus or clarify it.
(3) Write down instruments correction operation and store the correction records.
(4) Use apparatus which is appropriate for the purpose.
(5) Maintain instruments properly and store maintenance records.
(6) Microapparatus such as analytical balance, thermometer, glassware, watch, pipettes are corrected and make appropriate manual of standard level.
(7) Conduct performance test if the apparatus can give full ability.

V.6.3 Analytical methods

(1) Write down home-made analytical methods completely and discuss their propriety.
(2) Approval has to be gained when analytical methods are changed.
(3) Use the latest analytical methods.
(4) Conduct analyses following defined analytical methods.

V.6.4 Standard reagents and reference materials

(1) Keep standards which are needed for experiments.
(2) Use standards of guaranteed purity, or the best ones available.
(3) Write down the methods of preparation of standards for calibration curves.
(4) Store standards and reference materials in containers with their names or codes written clearly in an appropriate manner.

(5) Compare new batches of standards with the old ones before use. Use prescribed grade of reagents during such an examination.
(6) Keep a copy of the purity certificate when standard reagents are purchased.

V.6.5 Quality control

(1) Make calibration curves for each experiment.
(2) Ensure performance is within the prescribed range in the case of using control charts.
(3) Analyse QC samples regularly by pre-determined methods. Record the values obtained and, if the values exceed the limits, correspond with appropriate treatment.
(4) Analyse samples at random to check that results compare with the originally determined values.
(5) Get good results by using well considered experimental plans. Satisfy comparison results of interlaboratory examination. Don't have marked problems. Do some treatment if function is not enough.

V.6.6 Control of samples

(1) Make a document control system which clearly shows the receipt of samples, confirmation of samples against request form items, procedure of analytical development, fate of samples etc.
(2) Stick labels on samples and store properly.

V.6.7 Records

(1) Make records in notebooks or worksheets covering examination dates, analysts, items of analysis, detail of samples, examination records, all calculation, data of analytical instruments (output data), calibration curves data etc.
(2) Write down in notebooks or worksheets with ink and correct mistakes by crossing out. Also leave analysts' signatures.
(3) Sign corrections if mistakes are corrected.
(4) Copy data or check calculations following procedures which have been determined by institutes.
(5) Problems shouldn't happen about a series of inspection of random samples. (For example, check about samples, inspection of all procedure about from sample receiving to producing reports)

V.6.8 Definition of terminology

(1) *Accreditation (Laboratory)*

An accredited laboratory is an institute which is formally acknowledged by a designated public organization as a laboratory which has met specific analytical performance criteria. Being an accredited laboratory means the laboratory has both technical ability and fairness as an institute (sometimes it just means having the technical ability). Accreditation is generally given when the laboratory passes an ability evaluation (a test) as a institute. Accreditation is regularly re-evaluated.

(2) *Accuracy (ref. Error and Uncertainty)*

Accuracy refers to the difference between the average of several analytical values (of concentration), or each analytical values, and the true value (guaranteed value). When the term of accuracy is applied to a set of multiple observations of value, accuracy is the sum of accidental and systematic errors or bias. It is desirable to show the range of results within confidence limits in order to show accuracy of results from a standpoint that results have a certain doubtfulness. The true value is included within the range of confidence limits.

(3) *Analytes*

The compounds or elements in samples or standards which are directly or indirectly determined.

(4) *Batch*

A group of samples which is treated at the same time in order to determine the same analytes.

(5) *Bias*

Systematic errors which are caused by analytical operations. Average bias of analytical values from true values.

(6) *Blank Analysis (Blank Determination) (ref. Reagent Blank)*

This term is used for the analysis of a blank or non-contaminated matrix. This term is also used widely to describe experimental operations which are conducted without samples. For example, blank analysis is undertaken by following the adopted method using all reagents, solvents and procedures that would be followed for the analysis of the sample, but either no sample or a

non-contaminated sample is used. The purpose is to check contamination levels during the analytical procedures..

(7) *Blank Matrix (Blank Sample, Blank Solution)*

A blank matrix has the same composition as samples but is does not contain the target compounds. at levels above their detection limits. Blank matrices can be prepared as solutions. In this case matrix solutions have the same composition as sample solutions but do not contain target compounds.

(8) *Calibration*

A concentration series or value set which is established under special conditions. The relationship between the true concentrations of standards and the values gained from the analytical instruments.

(9) *Certified Reference (Matrix) Material, CRM (ref. Reference Material)*

Reference materials which are certified as containing specified concentrations of target compounds. They are produced by having independent laboratories using either the same, similar or different methods obtain specified analyte values. Certified values have always confidence limits.

(10) *Check Sample (ref. Reference Material, Quality Control Sample)*

Samples which contain target compounds of known concentration and whose compositions are similar to target samples. Check samples are analysed along with samples for quality control. In general check samples are made in-house and not appropriate for long term storage.

(11) *Collaborative Study*

Research in which multiple analytical institutes prepare, analyse and evaluate the same samples by the same analytical methods in order to confirm adequacy of the analytical methods. Also this term can be used as substitute for Interlaboratory Study.

(12) *Contamination*

In trace analysis, contamination is the unintentional mixing of the sample with target compounds or other compounds which then cause analytical interference and errors. Contamination tends to happen during analytical operations. To check whether there is contamination or not, blank analysis or analysis of reference materials are conducted as quality control.

(13) *Control Charts (ref. Quality Control Charts)*

(14) *Error (ref. Uncertainty, Accuracy)*

Analytical results, even the best analytical results, contain errors. These errors are the difference between true values and the analytical values. Errors include systematic errors and random errors.

- systematic errors: definite difference from true values when multiple analysis are conducted under the same conditions; errors which change the determined value from the true values according to a certain ratio when conditions are changed.
- random errors: irregular scatter which cannot be estimated when multiple analyse of the same amount are conducted under the same conditions.

For trace analysis, it is desirable to estimate the uncertainty of the results because true values are rarely known.

(15) *Interferent*

A constituent in samples which affects analysis measurements and results.

(16) *Interlaboratory Study*

A series of measurement in which a given set of samples is analysed independently at multiple analytical institutes. This term is sometimes used as substitute for Collaborative Study, and the study is conducted as a 'round robin' or 'ring test'.

(17) *Limit of Detection*

The detection limit, or limit of detection, of an analytical method is the least amount of target analyte in a samples which can be detected. The limit of detection is not the smallest amount of analyte for which the true value can be determined (see below). Detection limits of the concentration C_L, or amount q_L, are calculated from the value of the least amount of analyte which can be detected, X_L, accompanied by an certain uncertainty. X_L is calculated by the following formula.

$$X_L = X_{bL} + kS_{bL}$$

X_{bL} is average value of the blank, and S_{bL} is standard deviation of the blank, k is a coefficient which is determined by necessary confidence limits. Detection limits which are generally used are $3S_{bL}$ or three times the S/N ratio.

(18) *Limit of Determination*

The limit of determination of an analytical method is the smallest amount of target compounds

in a samples which can be determinate (measured) for a given uncertainty. It is also Limit of Quantitation, and it is effectively 10 times the S/N ratio.

(19) *Linearity*

Linearity of analytical methods means that relationship of concentrations and the signals obtained from the instruments follow a linear regression of the form y=mx+c in a certain range. m, c are coefficients.

(20) *Method (ref. Standard)*

All systematic operations which are used to undertake an analysis. This includes not only last measurement but all of the procedures related to analysis.

(21) *Precision (ref. Reproducibility, Repeatability)*

The degree of agreement of the results of measurements obtained from multiple examinations under the designated conditions.

(22) *Qualitative (ref. Quantitative)*

The term qualitative analysis means chemical analysis which is devised so as to confirm the components of a material or mixture, but which does not determine the exact quantity of components in the material or mixture.

(23) *Quality Assurance*

Systematic and intentional work which is undertaken to give definite reliability to analytical results.

(24) *Quality Control*

Specified work which institute staff undertake during analytical operations or measurements in order to judge if the analytical values are sufficiently reliable.

(25) *Quality Control Chart*

Graphs which are made from the results of measurements of Quality Control Samples which are prepared to check the reliability of the analytical results.

(26) *Quality Control Sample (ref. Check Sample)*

Samples of known concentration and similar composition to sample matrix. It is analysed with samples as an internal quality control.

(27) *Quantitative*

The term quantitative analysis means chemical analysis which is devised to determine numerical data values for one or more components of a material or mixture to specified certain confidence limits.

(28) *Range*

This term has two meanings.

1. the range of concentrations, from the smallest to the largest, of target compounds in samples to which the analytical methods can be applied.
2. the difference between the smallest and the largest values of data.

(29) *Reagent Blank (Solvent Blank) (ref. Blank Analysis)*

A Reagent Blank is a solution obtained after conducting all of the analytical process without using samples.

(30) *Recovery*

Recovery of a compound when more than one analytical procedure is conducted.

(31) *Reference Material (ref. Check Sample)*

This is a stable material in which the concentrations of one or more component is well established, and which is used for the confirmation of the calibration curves of analytical instruments, evaluation of analytical methods, or designation of the value of a material.

In the field of trace analysis, reference materials are briefly categorised into two types of reference materials.

1. Chemical substances of known purity that are used for the preparation of analytical standard solutions and calibration curves.
2. matrix standards : materials with the same or similar compositions to the analytical samples and in which the concentrations of target compounds are known. Matrix standards are used for the development of analytical methods, confirmation of the adequacy of the analytical methods, and comparison of results between laboratories or methods.

(32) *Repeatability (ref. Precision)*

The term Repeatability (r) refers to the range of values resulting from the difference in results when two samples are analysed by the same analytical method under the same conditions (by the same analysts, apparatus, laboratory and using the same experimental duration). Use 95 % as probability when there is no specific instruction.

(33) *Reporting Limit (ref. Limit of Detection, Limit of Determination)*

The Limit of the values of the analytical results which can be reported. Usually this is determined by agreement between analysts and their clients?.

(34) *Reproducibility (ref. Repeatability)*

The term Reproducibility (R) refers to the range of values resulting from the difference in results when two same samples are analysed by different analytical methods under different conditions (by different analysts, apparatus, and laboratory). Use 95 % as probability when there is no specific instruction.

(35) *Robustness*

The term Robustness refers to the sensitivity, or lack of sensitivity, of the analytical operations i.e. the ability of the parameters of the analytical methods to not be affected by trivial things. This becomes index of reliability when general samples are analysed.

(36) *Selectivity*

The degree to which non-target compounds affect the measurement of the analytical target compounds for a given analytical method.

(37) *Signal-to-Noise Ratio (S/N ratio)*

The ratio of the intensity of a controlled signal to the background instrument noise. Usually this value is the signal divided by the standard deviation of the background signal. (ref. Limit of Detection, Limit of Determination)

(38) *Spiked Samples*

"Spiking samples" is a common term used to imply the addition of solutions of target compounds of known concentrations to sample itself or to a matrix which is similar to the samples. (This is also called fortification of samples.)

(39) *Standard (all types)*

Standards are materials which are established by the acknowledged agreement of designated organizations. As for use of this regulation, standards are applied to materials, solutions (for example, organic compounds and metal solutions whose purity is known), and documents (for example, analytical methods and quality control system).

In this manual the following terms are used as standards.

- analytical standards (standard solutions) : solutions and matrices which contain target compounds which are used to confirm analytical methods or the ability of instruments.

- standard solutions for calibration curves : solutions and matrices whose concentrations are known, and which are used for making calibration curves from the response of instruments.
- internal standards : compounds which are spiked into samples, and have similar characteristics to target compounds and which are analysed together.
- external standards : generally target compounds are used, and analysed separately from samples.
- standard analytical methods : a series of written operational procedures for conducting chemical analysis, and which are acknowledged by a formal organisation.

(40) *Stock Solution*

Stock standards are solutions of standards or the sample prepared in relatively high concentrations and whose stability is well known. Standard solutions are prepared by diluting small amounts of stock solutions.

(41) *Sub-sample*

A part of the sample which represents the whole.

(42) *Traceability*

The term traceability refers to the ability to relate analytical data to appropriate standards such as international or domestic reference materials.

(43) *Ultra Trace Analysis*

This term, Ultra Trace Analysis, generally means analysis of concentrations less than 1 mg/kg, or 1 mg/L.

(44) *Uncertainty (ref. Accuracy, Error)*

Data has a certain range. True values exist at the confidence limit level within the range.

(45) *Validation*

This is the process which measures the ability of the chosen measurement operation to perform the desired task, and confirms if the method is capable of reaching a pre-determined level.

V.6.9 Check List

V.6.9.1 Check List Example of recording documents

a) Record (logbook) of entry to and exit from the analytical centre (room)

Date	Name	Time In	Time Out	Work Undertaken	Signature

*Logbooks should be located at the entrance of key rooms, such as sample storage room, clean room, etc.

b) Instrument logbook

Date	Name	Start Time	Finish Time	Sample Contents	Sample Numbers	Qualitative/ Quantitative	Comments (instrument condition etc.)	Signature

c) Sample storage logbook

Date	Name of person who brought sample	Sample Name	Numbers Weights	Sample state Liquid/solid /etc.	Container No.	Storage section	Comments	Signature

*Logbooks (use, preparation, and storage record) should be made for reagents, standard solution, and standard substances.

V.6.9.2 Instrument check list

a) Before use

Contents		Tick mark
1)	Did you record all details in the logbook?	( )
2)	Are the instruments and room tidy?	( )
3)	How is room air status?	(room temp. °C, humidity %)
4)	Ventilation duct	( )
	Power supply line	( )
	Gas supply line	( )
	Cooling system line	( )
	Other connected lines	( )

b) While running

Contents		Tick mark
1)	Any problem at start up?	( )
2)	Is OS working normally?	( )
3)	Is base line signal (blank intensity) normal?	( )
4)	Standard solution for calibration curves	
	Name of operative who prepared the solution	( )
	Date on which solutions were prepared	( )
	Type and numbers of standard solutions	( )
	Concentration range	(~ ppm)
5)	Analytical samples	
	Name of client	( )
	Sample name	( )
	Sample numbers	( )
	Sample condition : comments about existence of suspended particles etc.	
	Required analytical level	Qualification,semi-quantitative, quantitative analysis, close analysis
6)	Cross check samples?	No / Yes (kinds :)
7)	Blank samples?	No / Yes (How many?)
8)	Effects of interferences?	No / Yes (Was level of interference checked? ( ))
9)	Stability of instruments?	( )

c)After analysis

Contents		Tick mark
1)	Was all analysis completed? (analytical samples, blank, standard solution for check, etc.)	( )
2)	Confirmation of data output, and saving of data	( )
3)	Are instruments and room tidy?	( )
4)	Check power supply and other lines.	( )
5)	Entry in logbooks	( )

V.6.9.3 Check list for data analysis

Contents		Tick mark
1)	Cross check between entry items of analytical samples etc. and data file names of analytical samples	( )
2)	Entry of analytical procedures	( )
3)	Calculation of detection limits and quantitative limits Calculation of upper limit allowance range?	( ) ( )
4)	Are data of standard solutions and check substances within allowance limits from start to end of analysis?	( )
5)	Examination of dispersion because of repetitive analysis of each data.	( )
6)	Decision on significant figures	( )
7)	Is it necessary to adjust data? (If yes, method of adjustment)	( ) (contents:)
8)	Confirmation of cross check between list of analytical requirements and results report.	( )
9)	Explanation, agreement and signature of manager of analyst	( )
10)	Storage of raw data, calculation data, note of calculation procedure, and report of last results.	( )
11)	If requested analysis, are explanations in the client's report appropriate?	( )

VI Environmental monitoring - the Japanese case

VI.1 Co-operation with local public bodies

There are 61 environmental research related institutes and laboratories belonging to local governments at the prefectural level and in designated cities, employing about 2000 researchers (**Table VI-1-1**). If we look at researcher numbers by area, each area from the Hokkaido-Tohoku area to the Kyushu-Okinawa area (excepting metropolitan areas) has around 300 researchers. The distribution is well balanced. On the other hand, the Japanese government's Environmental Agency has two institutes and about 200 more researchers. This latter figure is about one tenth of the number of researcher employed by local governments. Therefore, it must be understood that researcher co-operation between government and local public organisations is extremely important when surveys of the distribution of harmful chemicals and/or continuous monitoring in Japan are conducted. For example, since 1974 the "Environmental Survey for Chemical Substances" has been conducted by co-operation of government and local public bodies. 752 chemical substances were investigated with 264 chemicals found in the environment by 1995. Of the chemicals detected, cautious essential chemicals (persistent chemicals and Class 1 Specified Chemical Substances based on the Chemical Substances Control Law etc.) which were judged to require continuous yearly monitoring are targeted for monitoring in water, sediment, and living things. In Japan, the Government regulates the sampling methods and analytical methods deemed suitable for target compounds, and is making efforts to build a monitoring network and maintain data reliability. Thus the results of the Government "fact-finding survey" gained in co-operation with local public bodies becomes information which is useful for environmental risk management and utilised to prevent health effect beforehand.

Table VI-1-1 Local institutes and researchers related to pollution / environmental issues (1995)

region	institutes	researchers
Tohoku, Hokkaido	10	250
Kanto Koshin-etsu	14	480
Chubu, Hokuriku	9	337
Kansai	8	299
Tyugoku, Shikoku	10	330
Kyushu, Okinawa	10	343
Total	61	2049
ref. Environmental Agency	2	211

VI.2 Facilities and instrumentation (Government and local public bodies)

Highly sensitive and accurate analytical instruments are necessary for the analysis of the chemicals designated in the priority list. In Japan, both common analytical instruments such as gas chromatography (GC) and large analytical instruments such as gas chromatography/mass spectrometer (GC/MS) are used at the same time to analyse multiple samples/multiple compounds. **Table VI-2-1** shows analytical facilities which are owned by local institutes. Seven institutes were chosen from 61 in whole Japan according to region, size, and researcher numbers. The analytical facility situation in Japan was estimated from this cohort of institutes. GC instruments can be allocated 4 - 7 researchers, High Performance Liquid Chromatography (HPLC) 10 -20, and Atomic Absorption Spectrometer (AAS) 8 - 12, and from such estimates it has been estimated that such instruments is to be found in each institute. At least one large instruments, such as GC/MS, which is indispensable for determination / quantitative analysis of large amount of harmful chemicals in the environment, is also found in each institute. The use of GC/MS is increasingly becoming a necessity, and in the future GC/MS it is expected to be as widespread as GC. ICP optical emission spectrometer and ICP/MS are rapidly becoming popular, and they will be found in every institute in the next several years. The Government is providing information and aid about which large instruments are desirable in the institutes of local public bodies.

Table VI-2-1 Main facility situation in the institutes of local public bodies (1996)

Institutes	A	B	C	D	E	F	G
Total stuff numbers	65	31	68	117	49	37	49
Researcher numbers	50 (19)	24	42	75	35	21	43
GC	8	6	20	13	7	6	11
GC/MS	1	2	6	7	6	2	6
HPLC	0	1	4	13	2	3	5
Ion Chromatography	1	1	2	1	1	2	2
AAS	4	2	2	3	5	3	2
Fluorescence Spectrophotometer	0	0	1	1	3	0	0
Infrared Spectrophotometer	0	0	1	1	1	0	1
Spectrophotometer	3	3	6	3	6	3	3
ICP Optical Emission Spectrometer	0	0	1	1	1	1	0
ICP/MS	0	1	1	1	1	0	1
Mercury Analyser	2	0	1	1	3	1	1
Element Analyser	0	1	2	0	1	0	0
Fluorescence X-ray Analyser?	0	1	1	1	1	1	0
NMR	0	0	0	0	0	0	0
Electron Microscope	0	0	1	2	0	0	2

note) Institute A has researchers in both hygienics and environmental research, ( ) shows the number of environment researchers.

VI. 3 Practical guidance and training systems

In Japan, an environmental survey of 1145 compounds in the new priority list based on a second general investigation of chemicals environmental safety has been conducted by Government in co-operation with local public bodies. Because of the targeting of large numbers of chemicals, the systematic training and upgrading of chemical analysis skills of teams of researchers and institutes had to be established. Several analytical training programs have been prepared in the Government and local public bodies. **Table VI-3-1** shows these training programs, which have been conducted by the Environmental Training Centre of the National Institute for Environmental Studies for the past three years. About 200 people per year, or 10 % of researchers in local public bodies, attended the training programs. The programs are prepared to meet the needs and levels of a wide range of participants, from the beginner to the advanced. Recently, programs covering the trace analysis of harmful chemicals using large instruments such as GC/MS or ICP optical emission spectrometer have been increasing. in addition, local public bodies also prepare training programs to train beginners and brush up the skills of the advanced.

Table VI-3-1 Training programs about environmental analysis conducted by the Environmental Training Centre of the National Institute for Environmental Studies

	program	target trainee	participant number		
			1993	1994	1995
1	instrumental analysis (general) (13 days)	Staff in charge of analytical methods related to pollution prevention with more than 1 years experience	43	47	42
2	general analysis (8 days)	Staff in charge of analytical methods related to pollution prevention with around 2 years experience	18	17	26
3	air quality analysis (13 days)	Staff in charge of analytical methods related to air quality or odour with more than 1 year experience	23	35	28
4	water quality analysis (13 days)	Staff in charge of analytical methods related to water quality, soil, or waste with more than 1 years experience	37	52	50
5	instrumental analysis (special) (3 courses, 5 days each)	Staff in charge of analytical methods related to pollution prevention with around 2 years experience			
6	theme analysis (3-4 courses, 5 days each)	Staff in charge of analytical methods related to pollution prevention	26	34	50
7	special analysis (21 days)	Staff in charge of analytical methods related to pollution prevention who have finished the Centre's analytical training courses or equivalent	1	1	1
8	environmental monitoring (water quality) (32 days)	Experienced management technicians in charge of water quality management in developing countries	11	10	10
total			189	224	247

Table VI-3-2 shows examples of local public body training schemes. Systems which provide and distribute new technology and information to the work place are well established. As mentioned above, local government and public bodies in Japan try to maintain reliability of data which are gained from environmental surveys of harmful chemicals.

Table VI-3-2 Examples of training of analytical technique in a local public body

	course	frequency (per person per year)	duration	contents
in-house	Training under-taken within research teams during normal, daily work routines	All the time	All the time	Team leader teaches group members to improve their techniques. Most important and efficient training.
	Academic paper reading and seminars	Several times	Regularly	By rotation group members charged with introducing key papers related to their research and giving seminars to share knowledge.
	Research report	Several times	As required	Report and discuss research progress and/or development.
	Training seminar reports	Several times	As required	Share information from seminars attended
	Practice lectures	Several times	A few days	Practice giving lecture to research group a few days before conference etc. Learn presentation methods and research problems.
External	Staff training at the training centre of local public bodies	0.5	A few days	Study the basic knowledge and general techniques needed by local public servants to conduct their work.
	Study program held by Government institutes	0.1	2 weeks to 2 months	Attend seminars about technique etc. held by Governmental institutes in order to develop and improve analytical skill and research ability.
	University research	< 0.1	1 year	Conduct specified research at university.
	Seminars	1 - 2	1 day	Learn latest technology by attending seminars held by analytical instrument companies.
	Conferences	2 - 3	2 - 3 days	Give presentation and exchange opinions at regular meetings and conferences of environment related academic societies.
	Study abroad	< 0.1	1 to 3 months	The researcher, after passing an examination to study abroad, undertakes research and training at an institute of choice
	Training on analytical instruments	0.2	1 week	Attend seminars held by instrument companies to learn operation and maintenance techniques for analytical instruments owned by researcher's institute.