Appendix C

Suggestion of Sampling Method for Environmental Surveys Concerning Chemical Substances

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O Environmental Survey (Water)

1. Sampling method

(1) Water

①Sampling time

The time for water sampling should be chosen when relatively sunny days have continued before the day of sampling and the water quality is stable.

② Sampling depth

The location for sampling should be in principle the surface water (0-50cm from the surface) in the center line of system of the surveyed point. However, water 1-2cm in depth should be avoided for sampling so that floating garbage and oils are not mixed in the samples.

⁽³⁾Preparation for analyzing

Supernatant removing garbage etc. should be used. In doing so, take care not to include the surface water. No filtration or centrifugal separation etc. is conducted.

(2) Bottom sediments

① Bottom sampling method

With consideration for the properties of bottom sediments, the bottom sediments collected with the Ekman-Birge bottom sampler or other proportionate bottom samplers should be placed in a clean tray and after removing extraneous substances such as pebbles, shells and bits of animals and plants, it should be provided to analysis after seaving with a 16-mesh seave (hole diameter 1mm). The sludge content (weight of sample through the seave / weight of original sample) (%) should be measured. Dry weight (105-110°C for about 2 hours) and ignition loss (600 ± 25 °C for about 2 hours) should be measured for part of the samples.

② Other points

Samples for analysis should in principle not be air or heat dried, and the measured value per dry weight should be calculated.

(3) Wildlife

① Samples

Samples should be fishes reproduced at the place of survey. In the sea areas, sea bass or young sea bass (if not available, goby, striped mullet or flatfish would do), and in the lakes, marshes and rivers, dace should be used (if not available, then carp or crusian carp would do) as standard samples. It would be desirable to use a single body for the samples, but use of several bodies is also possible. However, small body one should be used after sufficient cleansing.

② Preparation for analyzing

(a) Fishes

Edible parts (muscles) should be used in fish samples. The part to be collected for samples does not matter, but more than approximately 100 g should be carved and homogenized for samples. In case of the fish that its body weight is under 100 g, edible parts of several fishes should be carved and homogenized. In case of small fishes, the quantity of 100 g should be collected by carving the muscles from several bodies and homogenized. (b) Shellfishes (in the case when fishes are not available)

In shellfishes, edible parts of the necessary quantity should be collected and homogenized for use as samples. In this case, sludge should be removed as much as possible. ③ Other points

For wildlife samples, lipid weight (%) should be calculated by the following method. Take 5g of the sample in a homogenizer cup, add 20 ml chloroform and 40 ml methanol and homogenize for 2 minutes. Further add 20 ml chloroform and homogenize for 2 minutes. Filter with buchner funnel and precipitate should be homogenized with 80 ml chloroform:methanol (1:1). Take the whole chloroform and methanol fraction into the separatory funnel, and add 60 ml distilled water and shake slowly. Collect the lower chloroform fraction and after drying using anhydrous sodium sulfate, evaporate the solvent using rotary evaporator and dry the residue using phosphorus pentoxide and measure weight.

2. Hints for sampling

(1) The primary purpose of this survey is to investigate chemical substances persisting in the environment, and make sure whether they persist more in the environment than usual. Thus points where surveyed chemical substances are being released (for example near the outlet for waste water of factories etc. where the substances are being manufactured or used, or near points where transportation facilities pass through etc.) and points directly affected by pollution should be avoided as points for sampling.

(2) Three samples should be collected in a range of 500 square meters as a unit in the survey for water and bottom sediments, so that they are collected in as wide spread a point as possible. In this case, sampling in bottom sediments should be a mixture of samples in 3 spots in equal quantities within the surrounding 50 m. In surveys for fishes, a collection of 3 samples in the point is sufficient (It will be desirable to collect extras for frozen preservation in case problems arise.).

3. Investigation items on the samples

(1) Water samples : temperature, color by observation of the eye, transparency and turbidity

(2) Bottom sediment samples: appearance, odor, foreign substance, depth of the water at sampling point, water content, ignition loss and sludge content

(3) Wildlife samples: standard Japanese vernacular name, length of body (excluding tail), body weight and lipid weight.

4. Storage etc. of Samples

Collected samples should be placed in bags or containers so that the samples would not elute or adsorb, and should be analyzed as soon as possible. When preserving them, they should be placed in refrigerators or freezers etc. to prevent change in quality.

O Environmental Survey (Air)

1. Sampling method

①Sampling time

Sampling should take place between September and November when the weather is stable, for 3 continuous days, once a day, beginning at 10 am in principle for 24 hours. ②Sampling method

Samples should be collected by adsorption to resin or glass fiber filter etc.

$\mathbf 2$. Hints for sampling

The points for sampling should be where the situation of air in the points can be grasped, and points strongly affected by a particular source or by transportation facilities etc. should be avoided.

3. Investigation items on the sampling

Weather, temperature, humidity, direction of the wind, velocity of the wind and surrounding geography and situation of roads at the sampling time

4. Storage etc. of samples

Follow the case for environmental survey (water).