

Appendix C

Suggested Sampling Method for Environmental Surveys Concerning Chemical Substances

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1. Sampling method

(1) Water

[1] Sampling time

Water sampling was conducted at a time when the days preceding the day of sampling had been relatively sunny and the water quality was stable.

[2] Sampling depth

The location for sampling was, in principle, the surface water (0–50 cm from the surface) in the centerline of the system of the surveyed point. However, water 1–2 cm in depth was avoided for sampling so that floating garbage and oils were not mixed into the samples.

[3] Preparation for analysis

Supernatant-removing garbage, etc. was used. In doing so, care was taken not to include the surface water. No filtration or centrifugal separation, etc. was conducted.

(2) Bottom sediments

[1] Bottom sampling method

With consideration to the properties, the bottom sediments collected with the Ekman-Birge bottom sampler or other proportionate bottom samplers were placed in a clean tray and after removing extraneous substances such as pebbles, shells and bits of animals and plants, and then sieving with a 16-mesh sieve (hole diameter of 1 mm), they were provided for analysis. The sludge content (weight of sample through the sieve / weight of original sample) (%) was measured. Dry weight (105–110°C for about 2 hours) and ignition loss ($600 \pm 25^\circ\text{C}$ for about 2 hours) was measured for part of the samples.

[2] Other points

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

(3) Wildlife

[1] Samples

Samples were those fish reproduced at the place of survey. In the sea areas, sea bass or young sea bass (if not available, goby, striped mullet or flatfish were accepted), and in the lakes, marshes and rivers, dace were used (if not available, then carp or crucian carp was accepted) as standard samples. It was preferable to use a single body for the samples, but the use of several bodies was also allowed. However, a small-bodied sample was used after sufficient cleansing.

[2] Preparation for analysis

(a) Fish

Edible parts (muscles) were used in fish samples. The part to be collected for samples did not matter, but more than approximately 100 g was carved and homogenized for samples. For cases in which the body weight of the fish was under 100 g, the edible parts of several fish were carved and homogenized. In the case of small fish, 100 g was collected by carving the muscles from several bodies, and then homogenized.

(b) Shellfish (for cases in which fish were not available)

For shellfish, the edible parts of the required quantity were collected and homogenized for use as samples. In this case, sludge was removed as much as possible.

[3] Other points

For wildlife samples, lipid weight (%) was calculated by the following method:

Five grams of the sample was placed in a homogenizer cup, after which 20 ml of chloroform and 40 ml of methanol was added, and then the sample was homogenized for 2 minutes. An additional 20 ml of chloroform was added, followed by 2 minutes of homogenizing. The sample was then filtered with a Buchner funnel and the precipitate was homogenized with 80 ml of chloroform:methanol (1:1). The entire chloroform and methanol fraction was placed in the separation funnel, after which 60 ml of distilled water was added and then the mixture was shaken gently. The lower chloroform fraction was collected and after drying with anhydrous sodium sulfate, the solvent was evaporated using a rotary evaporator. The residue was dried using phosphorus pentoxide, and the weight was measured.

(4) Air

[1] Sampling time

Sampling took place between September and November when the weather was stable, for 3 continuous days, once a day, beginning at 10 a.m., in principle, for 24 hours.

[2] Sampling method

Samples were collected by adsorption to resin or glass fiber filters, etc. or were sucked by canister.

2. Sampling sites

(1) The primary purpose of this survey was to investigate the persistence of chemical substances in the environment, and to determine whether they persist in the environment more than usual. Thus, the points where surveyed chemical substances were being released (for example, near the outlet for waste water of a factory, etc. where the substances were being manufactured or used, or near points through which transportation facilities passed, etc.) and points directly affected by pollution were avoided as points for sampling.

(2) Three samples were collected within a range of 500 square meters as a unit in the survey for water and bottom sediments, so that they were collected in as widespread a point as possible. In this case, the sampling for bottom sediments was a mixture of samples from 3 spots in equal quantities within the surrounding 50 m. In the surveys for fish, a collection of 3 samples from the area was considered sufficient. (It was preferable to collect extra samples for frozen preservation in case a problem should arise.)

(3) The points for air sampling were where it was possible to grasp the status of the air. Points strongly affected by a particular source or by transportation facilities, etc. were avoided.

3. Investigation items on the samples

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

(2) Bottom sediment samples: appearance, odor, foreign substance, depth of 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) Air samples: Weather, temperature, humidity, wind direction, wind velocity and surrounding geography and status of roads at the sampling time.

4. Storage, etc. of samples

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