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A. SUMMARY AND CONCLUSIONS

1. A Test Guideline has been established for determining the potential of a chemical to taint seafood. This Guideline is based on the exposure of fish and the evaluation of the imparted taint by a triangular test.
2. The Test Guideline has been evaluated in a small ring test in which five laboratories tested four chemicals with three fish species.
3. Based on the limited results of the ring test, none of the following factors appear to have a systematic effect on the evaluation of the potential to cause taint :
 - a) the performing laboratory;
 - b) type of water used (sea- or freshwater);
 - c) fish species;
 - d) evaluation by flavour or by odour;
 - e) method of preparation and presentation of samples for sensory evaluation.
4. One laboratory tested four chemicals following the proposed Test Guideline and the GESAMP guideline. Concordant results were obtained.
5. The proposed Test Guideline also contains a procedure for estimating the loss of taint from the test species during a depuration phase in clean water.
6. The hazard that a chemical will, after an accidental spill from a chemical tanker, taint seafood, depends not only on the intrinsic property of the chemical to cause tainting but also on the exposure of the seafood to the chemical. The probability that the tainted seafood will be marketed is determined by the likelihood of a spillage occurring, the intrinsic tainting property of the chemical, the exposure conditions and the likelihood of the tainted seafood to be caught or harvested. Calculations indicate that this probability is minimal under normal conditions.

B. INTRODUCTION

The value of recreationally and commercially important seafood might be reduced by the introduction of tainting compounds into the sea. Tainting compounds are taken here to be chemical substances which when taken up by aquatic organisms, result in a flavour or odour that is not typical of the species.

The IMO/FAO/UNESCO/WHO/IAEA/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP) considered the possible tainting of sea food by chemical substances to be an important issue. When evaluating the hazards of harmful substances carried by ships, the potential of a chemical to cause tainting is one of the parameters used by GESAMP in establishing the hazard profile of the chemical. Under the MARPOL convention (the International Convention for the Prevention of Pollution from Ships, held in 1973) the hazard profile is used by IMO (International Maritime Organisation) to allocate the ship type in which the chemical should be transported by sea (GESAMP, 1982).

A number of authors have reviewed the literature dealing with off-flavours in fish caused by chemicals (Shumway, 1970; Shumway and Palensky, 1973; Stansby, 1978; Persson, 1981, 1984). In these reviews little attention has been paid to critical assessment of the test methods used. This is striking since taste evaluations are inherently subjective and the test method might be expected to strongly influence the test result. Although some standardisation has been attempted for specific purposes (ASTM, 1978) no generally accepted test method is available which defines the main components of taint assessment : the fish exposure conditions, the fish flesh preparations, the sensory evaluation by a panel and statistical treatment of the results.

Recognising this, GESAMP recently developed "draft guidelines for evaluating threshold values for fish tainting" (GESAMP, 1983) thereby stimulating efforts to develop generally acceptable standard test guidelines.

Having established guidelines for taint testing it is important to be able to evaluate the likelihood that a chemical with the potential to cause taint will in fact taint fish in the marine environment as a result of accidental spills or the discharge of tank washings.

In view of the requirement for a more detailed protocol for determining the potential for taint and the need to consider this potential in relation to the fate of chemicals in the sea a Task Force was set up. The Terms of Reference were :

1. To develop within the framework of the GESAMP guidelines (1983), a test guideline for determining the concentration of a chemical in water which taints the flesh of fish and, in addition, the subsequent effect of depuration.
2. To verify the test guideline experimentally and to modify it, as necessary, in the light of the results.
3. To consider in relation to the threshold tainting concentration the biological, physical and chemical factors which should be taken into account to assess the likelihood that a chemical will taint fish in practice.

C. BACKGROUND

Ships which transport chemicals in bulk by sea might introduce them into the marine environment as a result of accidents or permitted discharges (e.g. tank washings). When a chemical with the potential to cause the tainting of seafood is introduced into the marine environment the hazard it poses with regard to the tainting of seafood should be established. Factors which have to be taken into account when assessing this hazard are : the inherent flavour or odour of the chemical, the amount of chemical which might be introduced and the biological, physical and chemical factors which determine the fate of the chemical in the sea and subsequently in the marine organisms.

This report considers the establishment of a test guideline and the interpretation of the results from fish taint tests, taking into account the above mentioned factors.

According to the GESAMP guideline (1983) the laboratory experiment should represent an "acute case" i.e. provide information about the tainting of

commercially important seafood organisms which might occur following a short term exposure.

The probability of a tainted organism being caught is lower with tainting compounds which are rapidly metabolised to non-tainting substances or which are rapidly excreted. The guideline developed here for determination of taint also allows for an assessment of the importance of depuration.

D. ESTABLISHMENT OF A TEST GUIDELINE

1. Introduction

The aim of the guideline is to provide a procedure for the evaluation of the potential of a chemical to taint seafood. Whilst the importance is recognised of the concept of a taint threshold value, that is of a minimum concentration of a substance in water which results in tainted seafood, neither the definition of taint threshold value nor the principles by which it can be measured are established. ECETOC has therefore confined itself to establishing a procedure which allowing categorisation of chemicals according to the potential to cause taint.

An assessment of tainting must include a period of exposure of the test organism to a test substance followed by determination whether taint is present. In many situations the organisms exposed to tainting chemicals will have an opportunity to depurate before consumption; therefore consideration was given to a procedure to determine whether depuration occurs.

In outline, a procedure for evaluating the ability of a chemical to cause taint involves exposure of a test-organism to a chemical under defined conditions, followed by sensory evaluation to determine if the exposed organism is tainted. Depuration is then tested by transferring some exposed organisms to water free of the test substance and re-evaluating of the organism for taint (cf. Appendix 1).

2. Exposure of Fish

2.1. Information on test substance

The substance tested should be the grade which is transported in bulk at sea. A knowledge of the solubility, density, melting- and boiling-points, and the vapour pressure of the test substance at ambient atmospheric conditions is required. This information can be used to predict the behaviour of the test substance during exposure of the test organism and hence to determine the nature of the test system required, e.g. static aerated, semi-static with or without aeration, continuous flow or sealed.

Information on the hazard of the substance to man will also be required since certain health hazards may preclude its evaluation by the method of test described here.

2.2. Test organism

The GESAMP guideline for the assessment of tainting (GESAMP, 1983) refers to the "liability of substances to taint seafood". A wide variety of animals and plants can properly be considered as seafood; clearly it is not possible to test all seafoods.

The three principal groups of seafood organisms are fish, crustaceans and molluscs. It is clear that in various respects crustaceans and molluscs differ from fish, but the latter was selected as the most suitable group on which to base a guideline for assessment of taint because :

- they are the major source of seafood;
- they are readily available for testing; many species are capable of being reared in the laboratory and some are farmed on a commercial basis;
- they are maintained relatively easily under laboratory conditions;
- they are used extensively in aquatic toxicology. More laboratories have experience with fish than with molluscs or crustaceans.

The use of a marine fish species was considered to be desirable but no generally acceptable species could be found. The main reason for this was the limited availability of species of a suitable size and the lack of data on the acute toxicity of chemicals to marine fish in general.

Rainbow trout (Salmo gairdneri Rich.) was chosen as the preferred test organism. This fish trout is widely available, it has a moderate fat content and can be relatively easily kept in the laboratory. Being a euryhaline fish it can live in either fresh- or sea water and, if necessary, can be adapted from one medium to the other. In some situations it may be difficult to use rainbow trout as a test species and in such cases other species such as red sea bream (Pagrus maior) or carp (Cyprinus carpio) are considered suitable. Since the primary purpose of the test guideline is the determination of the taint level of chemicals transported in bulk by sea, sea water is the preferred medium, but the guideline can be used with freshwater.

Considerable evidence indicates that the fat concentration in fish may influence the accumulation of certain chemicals in the flesh. To ensure the comparability of data the fat content of the edible portion of the fish to be used for testing should be determined and lie in the range 3-10%. There are a number of suitable methods for determining the fat content (cf. Appendix 2).

The fish should not be fed immediately prior to or during the test since this removes the possibility of uptake of taint from test substance adsorbed onto the food and reduces the faecal contamination of the water. Knowledge of the previous diet of the fish is important in ensuring that no strong or atypical flavours result from the diet and mask the taint from the test chemical. If mortalities occur the test conditions should be reassessed.

2.3. Duration of exposure

Choice of the duration of exposure must inevitably be a compromise. In the environment some organisms might be exposed to high concentrations for short periods, others to low concentrations for long periods. It was considered that in general an exposure period of 24h, as recommended by GESAMP (1984), was acceptable.

2.4. Exposure concentrations

An initial test, or limit test, should be carried out at a concentration of 10 mg.l^{-1} , or 1/10 of the 24h LC_{50} , or the limit of water solubility level whichever is the lowest. For most chemicals a concentration of 10

mg.l⁻¹ was considered to be the maximum concentration likely to be sustained over a period of 24h in the environment following an accidental or operational discharge from a ship (cf. Chapter F). The alternatives, of testing at 1/10 of the 24h LC₅₀, and the limit of water solubility concentration, are included to ensure that few if any of the exposed organisms will die during the test and that only concentrations below the water solubility are tested.

If taint is detected in the limit test further testing at at least two supplementary exposure concentrations (1.0 and 0.1 mg.l⁻¹) should be carried out. When taint is detected in the limit test the depuration should be used to assess the persistence of the taint.

Analysis of the test substance in the exposure medium at the beginning and at the end of exposure is necessary to check the stability of the concentrations throughout the test. The exposure concentration of the substance at the end of exposure should be within \pm 50% of its initial concentration.

The use of co-solvents should be avoided if at all possible but they may be necessary when making up stock solutions of substances with low water solubility. If a co-solvent is used, evidence must be available to show that it is not capable of imparting taint to the test organism.

2.5. Test conditions

The preferred exposure medium is sea water and the fish should be acclimatized to it so that exposure to the test chemical does not occur whilst the fish is in a stressed condition. Acclimatization of Salmo gairdneri of the size required for testing can be achieved. The salinity of the sea-water should be 32-36 o/oo , encompassing the range typical for the open sea. Either natural or an artificial (reconstituted) sea-water is acceptable. In both cases care should be taken to ensure that the batch of water used does not itself possess any potential for taint. This should be checked by smelling the water and tasting some of the control fish before the experiment. Its detection at an earlier stage will avoid unnecessary expenditure and delays. In all tests a batch of control fish should be held under the same conditions as the exposed fish except that no test substance is present.

Continuous flow tests are considered preferable although semi-static or static tests are not excluded. Fish should not be stressed by overcrowding; the concentration of dissolved oxygen should remain above 60% of the air saturation value throughout the test. The water temperature should be controlled at $15 \pm 2^{\circ}\text{C}$. Other fish species may need to be kept at a different temperature. The pH of the sea water is unlikely to vary significantly during the test but initial and final pH values should be recorded. It is advisable to check that concentrations of nitrite nitrogen in the water are less than 0.1 mg.l^{-1} as concentrations in excess of this might cause mortality of Salmo gairdneri.

3. Uptake and persistence of taint

GESAMP (1982) stated that "where a substance causes taint it would be given a "T" rating even though it is known to have a relatively short half-life in the animal". Although GESAMP knew that substances can depurate from animals, they decided against the inclusion of a depuration phase in the assessment of taint. The purpose of a depuration phase is to allow an assessment of taint retention once exposure has ceased. Depuration might occur when free swimming organisms move out of the contaminated zone, when the contaminated water itself moves away from the zone the organisms inhabit, or when the compound itself rapidly decomposes. The mechanism of depuration can be passive physical loss, active metabolism or a combination of both.

GESAMP's decision not to include a depuration phase was made because they considered that "though absorption of chemicals is rapid, depuration is very slow" (GESAMP, 1984). There is, however, sufficient evidence in the literature to question the validity of this statement. Numerous experiments with different chemicals including a number known to impart taint to fish, have demonstrated a short "half-life" time for residues in fish tissues. It is, however, accepted that depuration will not always be rapid. The uptake and persistence of a chemical in the fish, and hence the persistence of taint, is dependent on :

- the concentration of the chemical accumulated during exposure;
- the rate of loss of the chemical due to metabolism;
- the rate of excretion of the chemical by active or passive means.

Experience has shown that the levels to which chemicals are accumulated under similar and constant conditions of exposure can differ greatly (Hamelink and Spacie, 1977). In addition, rates of depuration, once exposure has ceased, can vary from substance to substance (Binder et al., 1984).

3.1. Uptake

It is now widely accepted that non-ionized substances with a log-partition coefficient for n-octanol/water ($\log P_{ow}$) greater than three have the potential to bioaccumulate to a significant extent in aquatic organisms (OECD, 1984).

The organs in which the substance accumulates, e.g. adipose tissue, muscle or liver may differ according to the nature of the chemical. Conell (1978) demonstrated a highly significant correlation between the percentage of accumulated hydrocarbons in muscle tissue and the percentage of lipids in the muscle tissue.

3.2. Metabolism

Fish possess the ability to metabolize many xenobiotic compounds (Dewaide, 1971). Many fish species have a complement of enzyme systems (hydrolytic, reductive conjugative and mono-oxygenase systems) (Fouremant and Bend, 1983) or show an activation of the enzyme systems capable of metabolizing xenobiotics (Elcombe et al., 1979).

3.3. Depuration

There are numerous examples of rapid depuration of chemicals from fish tissues. Veith et al. (1980) reported half life values of less than 24h for 16 out of 25 chemicals to which fish had been exposed for periods of 14-28 days. Persson (1984) reported the uptake and depuration behaviour of six chemicals and for three of these, purging times for the taint was 24h or less. Rapid depuration of naphthalene and methylnaphthalene was also recorded by Korn and Rice (1981). Branson et al. (1979) showed that fish tainting residues of diphenyl oxide were reduced by 50% each day. They also found a significant correlation between fish taint and the level of residues in the fish flesh. Ogata and Miyake (1978) found the half-life of benzene, toluene, m- or p-xylene and o-xylene to be 0.5, 1.4, 2.6 and 2.0 days respectively. However there are cases where the depuration times are

long, e.g. 2,5,2',5'-tetra-chlorobiphenyl has a half life of 1.76 years (Binder et al., 1984, Persson, 1984).

In light of the above evidence showing that relatively rapid depuration may be a common phenomenon it was thought desirable to include a depuration phase in the test procedures to be followed.

3.4. Comparison of Freshwater and Marine Fish

No evidence is available to suggest that different accumulation or depuration processes for chemicals occur in marine- and freshwater fish. On the contrary, investigations of toxicity and bioaccumulation with fish from both environments have given similar results (Niimi, 1983, Dawson et al., 1975/1977, Zarogian et al., 1985). Further comparative data on the uptake and loss of tainting chemicals in fresh- and sea water fish are, however needed.

4. Sensory Evaluation of Taint

4.1. Preparation of fish for evaluation

The edible portion of fish gradually change in flavour on storage. If stored under refrigeration these changes are small over the first 48 h and will not affect evaluation of taint. Fish is prepared for cooking and subsequently cooked. This in itself imparts flavour or odour to the product. This potential problem can be overcome by the use of adequate controls and the arrangements of the triangular test. Suitable procedures for cooking include cooking in closed containers, in steam or boiling water, or by microwaves. The edible portion of the fish is then presented to the panel for sensory evaluation.

4.2. The choice of the triangular test for detecting taints

The experimental procedures described in these guidelines are intended to determine whether or not fish are tainted following exposure to chemicals in the water in which they are held. In this context taint is any flavour or odour foreign to the fish (ISO, 1983-a) and is not used in the more restricted meaning of an unpleasant flavour or odour.

Taint is a subjective experience and can only be evaluated by human sensory organs. A suitable procedure for evaluating whether or not a sample is tainted was considered to be the triangular test. In this

procedure an assessor is presented with 3 samples, 2 are identical (either test or control material) and the third is of the other treatment. The assessor is required to identify the single specimen from the pair. So long as the experimental procedure as laid down by ISO (1983-b) is followed, the test is free of bias. Appendix 3 gives more details why this technique was chosen.

4.3. Selection of assessors

On the basis of the principles described in Appendix 2, a panel of 15-20 people typical of the general population of consumers is recommended. The assessors should have some familiarity with the triangular test procedure and the normal flavour or odour of the species of fish used in the test. They should not be specially selected for acuity to the chemical under test.

There is a possibility that the assessment by the panels may differ consistently between and within laboratories and change as they become more experienced. These systematic differences are, however, likely to be small in comparison with other sources of variation arising from the experimental procedures.

4.4. Evaluation of results from a triangular test

The triangular test provides data which is amenable to statistical analysis. Detail instruction is given in ISO 4120 procedure (ISO, 1983-b). A discussion of the statistical principles involved is included in Appendix 2.

E. VALIDATION OF GUIDELINE

1. Participants in the Ring-test

Four West-European laboratories (3 industrial and 1 governmental) and one Japanese "contract" laboratory participated in the ring-test. Three laboratories performed both the exposure and the taint assessment phases. The other two laboratories only carried out the exposure of the fish to the chemicals and used specialised contract laboratories for the taint assessment.