Addendum to the Environmental Risk Assessment for tert-Butyl methyl ether (MTBE)

CAS No.	1634-04-4
EINECS No.	216-653-1
IUPAC Name:	Propane, 2-methoxy-2-methyl

1 Introduction

The environmental risk assessment of MTBE was completed with a conclusion (i) for the aquatic ecosystem (ECB 2002). There was a need for better information to adequately characterize the risks to the aquatic ecosystem regarding the emission of the substance to surface water. The information and test requirements were: a tiered testing approach for investigation of avoidance behaviour in fish and if necessary in other wildlife animals related to water contaminated with the substance.

It was agreed at the Technical Meeting to perform an Eels (Anguilla anguilla) Avoidance Test with MTBE as a screening test. The aim of the study was to examine if there will be environmental, in this case, avoidance, effects at a concentration of 30 µg/l, which is the concentration threshold for fish tainting effect. In case there will be no avoidance behaviour at the concentration of 30 µg/l, not further testing is needed and conclusion (i) can be changed to conclusion (ii). If avoidance behaviour would be observed at 30 µg/l, the Rapporteur would draft a report discussing the relevance and use of the result. The Technical Meeting also decided that, irrespective of the result of the screening test, no further studying would be requested within the ESR program.

This Addendum contains the summary of the fish tainting study (Petersen & Møller 2001) which is the basis for the avoidance test concentration and the summary of the eels avoidance behaviour test (Petersen 2003). The subsequent conclusions for the risk assessment and the revised risk characterisation are also included.

2 Fish Tainting Study

The measurement of the capacity of MTBE to cause taint in fish when present in the ambient water was conducted in two stages:

1. exposure of the organism to the chemical
2. evaluation of the exposed organism for taint.

The test follows the principles described in the ECETOC guideline (ECETOC 1987).

Rainbow trout *(Oncorhynchus mykiss)* were exposed to MTBE at the nominal concentrations: 20, 40 and 80 µg/l plus 3 control groups. For each concentration and control group 20 fish were exposed for a period of 14 days. The test was semi-static and the test water was renewed three times a week. Measured concentrations were determined from a freshly produced test solution (t=0h) and from the respective 3 days old test

solution (t=72h). At the end of the exposure period, fish were harvested and killed. The taint testing was started less than 4 hours after harvest of the fish.

The fish were skinned and filleted and visible and noticeable fish bones were removed. The fillets were cut into pieces of same size. The pieces were heated 4.5 minutes at approximately 100 ºC after which the temperature in the center of the fillets was approximately 70 ºC. The fish were served to the assessors immediately.

The examined effect is taint, which is a sensory experience that can be directly evaluated by a sensory discrimination test as the Triangular test. In the Triangular test method, the assessors are simultaneously presented to a set of three samples, two of which are identical and one, which is diverging from the two others. In the test, the assessors have to identify the diverging sample. Each assessor evaluated six sets of samples from each MTBE exposed group totalling 48 test results per exposure concentration. The Triangular test is a forced choice test, in which the assessor has to make a decision about the diverging sample. The applied method has been used according to the Triangular test procedure, for which an international standard is available (ISO 4120). The test results are subsequently processed to give a tainting threshold concentration.

The assessors of the actual test panel have completed a basic training course according based on ISO 8586-1 and ASTM STP 758.

The mean measured concentrations in the test water were 15, 31 and 56 µg/l. The results of the Triangular test can be seen from Table 1.

Table 1 Results of the Triangular test

MTBE concentration (actual concentration)	15 µg/l	31 µg/l	56 µg/l
Number of assessors	8	8	7
Number of assessments	48	48	42
Number of correct assessments	15	22	22
Significance levels	Ns	*	**
Diverging sample chosen because of odour	7	10	10
Diverging sample chosen because of taste	13	22	18
Comments	None	None	None

Significance levels: ***Significance of 0.1% level; **Significance of 1% level; *Significance of 5% level; ns -No significance.
Significance levels are calculated on the basis of binomial distribution.

It can be concluded from the results that it is possible to taste MTBE in fish exposed to an actual MTBE concentration of 31 µg/l at a significance level of 5 %. It was not significantly possible to distinguish fish exposed to 15 µg/l MTBE/l.

3 Fish Avoidance Study

3.1 Test performance

The study was initiated in order to investigate a potential avoidance behaviour in juvenile stages of eel *(Anguilla anguilla)* exposed to a MTBE concentration of 30 µg/l in water. The test substance was 99.9% MTBE and the reference substance was zinc sulphate. The test was conducted in compliance with the OECD Principles of Good Laboratory Practice.

The test was carried out in a flow-through vessel equipped with a video monitoring system. The principle of the test system is to create two parallel currents without physical separation. The two currents consist of clean water and test water, respectively. The eels are free to move between the two currents. The distribution behaviour of the exposed eels is observed by video monitoring under conditions, at which possible external stress factors are minimized.

The test was performed with active carbon-filtered tap water with a temperature of 15 ± 1 ºC and pH 7.5 ± 1.

Eels were chosen for test organism due to their general high activity pattern which is an advantage in the avoidance test system. Since MTBE is very volatile from water, it was stressed in the test protocol that a pre-test without eels would be performed in order to verify that the actual test concentration is close to 30 µg/l. The actual test concentrations were measured.

Before starting the test, the laminar flow of the test system was checked in a colour experiment, in which MTBE (30 µg/L) and colour (Na-fluorescein) was added to the test water. The flow rate and placing of the sponges were then optimised and the observation area was divided into three zones (clean water, zone with MTBE in water and mixing zone).

The test consisted of three interlinked studies. The first part was the control-control study, the second part formed the MTBE impact study and the third part formed the reference study. Each of these three parts comprised two observation periods plus an acclimatisation period and an interim phase. The control study as well as the impact study was performed with a total of 10 replicates whereas the reference study was performed with 5 replicates only. For each replicate, six fresh eels were used.

Control-control study consisted of an 10 minute acclimation phase, control phase A, interim phase and control phase B. At the end of the acclimation phase, the video recording was started and the distribution of the eels in the observation area was recorded every 15 seconds for an 8-minute period. Interim period lasted 9 minutes and compensated for the time needed in the impact study to allow the MTBE concentration to be established. After the interim period the video recording was started again and the recording was made as in control phase A. The control phase B compensated for any time-dependent deviations and shifts in behaviour.

The outline for the impact study was similar to that of the control-control study except for one point. After the control phase MTBE was conveyed into the system. After the interim phase, the recording of the presence of the eels in the impact zone was started

(impact phase). The distribution of the eels in the observation area was recorded every 15 seconds for an 8-minute period both at control phase and impact phase.

In order to test the sensitivity of the eels to chemical impact, a reference study was performed with zinc sulphate at a concentration of 10 mg Zn^{++}/L and 30 mg Zn^{++}/L, respectively.

The statistical analysis performed was the chi^2 test at the 5 % level with one degree of freedom.

3.2 Test results

The colour control test showed that, both with and without MTBE, the mixing zone area was only minor (<12%).

The results of the chemical analysis showed that MTBE concentration in the control phase varied between 0.03 and 0.19 µg/L, with a mean value of 0.08 µg/L. The concentration in the control zone during the MTBE impact studies varied between 0.03 and 0.23 µg/L with a mean value of 0.10 µg/L. The MTBE concentrations in the impact zone varied between 2.9 and 22 µg/L with a mean value of 15.76 µg/L (STDEV=5.78).

In the reference study the actual concentration with nominal 30 mg/L of zinc sulphate was 24 mg/l in the beginning of the observation period and 15 mg/l at the end of the observation period.

It can be concluded from the results of the control-control study that there was no significant difference in the distribution of the eels before and after the interim phase.

There was a significant difference in the distribution of the eels between the control-control study and the MTBE impact study. The distribution results show that the eels do not avoid MTBE. The eels were more present in the MTBE impact zone than in the clean-water zone.

In the reference test the presence of eels both in the Zn^{++} 10 mg/L and 30 mg/L impact zone was significantly lower than the control zone.

3.3 Test validation

According to the test report the test design is considered valid as the following validity criteria are fulfilled:

- The colour tests proved a laminar flow
- The distribution in the control-control experiments showed that the distribution over time was equal. There was no significant difference in the distribution of the eels before and after the interim phase.
- The reference studies showed that the presence of eels in the Zn impact zone was significantly lower than the control zone. The test design can thus be assumed to react adequately in relation to avoidance reactions.

3.4 Discussion

Eels have an extremely sensitive olfactoric system. The feeding behaviour of the adults is based on this ability to detect traced of organic molecules in water. Attraction to organic molecules is a natural behaviour in eels. In their migration from seawater into river basins, young eels also positively orientate themselves via their olfactoric system. Furthermore, the test system described in the present report has clearly demonstrated an avoidance reaction of eels with zinc sulphate.

From the obtained results, the eels do not seem to avoid MTBE exposure at the concentration tested. A preference towards the MTBE-exposed part of the test chamber was observed. The feeding strategies of eels are based on a preference behaviour towards organic molecules. These serve as indicators for decaying organic material, the natural food source for eels. This natural behaviour might explain the MTBE preference and the fact that a sort of grouping of the test specimen can be observed. This grouping is very obvious in the MTBE study but also appears in the pure control study.

4 Conclusions

The purpose of the study was to investigate if eels would show avoidance behaviour at a MTBE concentration of 30 µg/L. Despite of the fact which was stressed in the test protocol, the need to verify that the actual exposure concentration is close to 30 µg/L of MTBE, the concentration in the study was significantly lower. The study gives an answer that eels do not show avoidance behaviour but instead preference towards MTBE at a concentration of 15.76 ± 5.78 µg/L. The 30 µg/L was chosen because it is the same concentration as the concentration in water when tainting of fish can be detected. An interest lies in the question if fish avoid the concentration where tainting is detected. Unfortunately, the study does not answer this question.

It can, however, be concluded that the test will not have to be redone in order to meet the needs of the agreed procedure. Prior to having the result of the avoidance study it was chosen by national authorities e.g. in Denmark to use the tainting limit value of 15 µg/l as a regulatory limit for surface water contamination. At this concentration there was no tainting detected. In case there would have been avoidance behaviour at a concentration of 30 µ/L further testing could have been necessary in order to establish a limit value for regulatory use. However, as the study shows, there is no avoidance behaviour at a concentration of 15 µ/L, thus the possible regulatory limit value for surface water contamination will not change following the result of the avoidance study. There might be some concern from the fact that eels show preference in the study to MTBE at concentration 15 µg/l. If they would show same behaviour at higher concentration, it could lead to tainting of fish. No conclusion can, however, be made from this laboratory study. It is obvious that the behaviour of fish in natural waters depends on several variables that are not included in the laboratory test. For example the higher organic matter content in natural waters compared to the tap water used in the test might affect the avoidance behaviour significantly.

As a conclusion the study meets the needs of the risk assessment despite the fact that it does not completely answer the specific question addressed. No further testing is needed and conclusion (i) can be changed to conclusion (ii).

5. Revised Risk Characterisation

OVERALL RESULTS OF THE RISK ASSESSMENT ADDENDUM

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Aquatic environment

( ) (i) There is a need for further information and/or testing

(X) (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion applies to the need for better information to adequately characterise the risks to the aquatic eco-system regarding the emission of the substance to surface water.

6 References

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