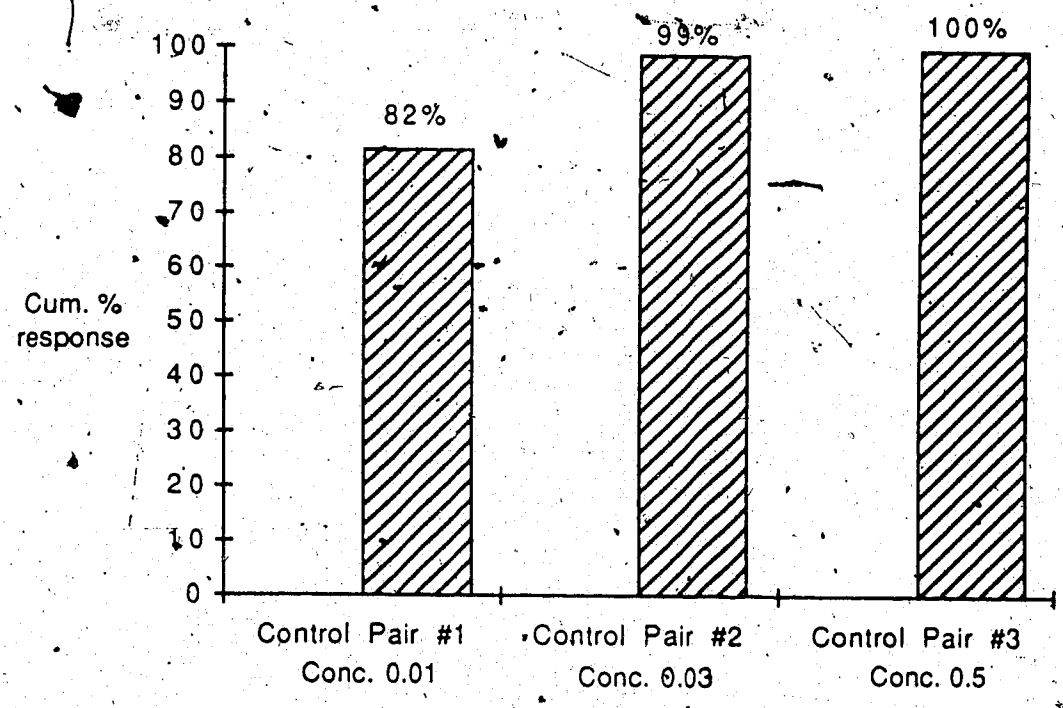

Figure 26. Sensory evaluation, replicate #1, cumulative percent response of exposed or fortified flesh vs control at each of three concentrations.

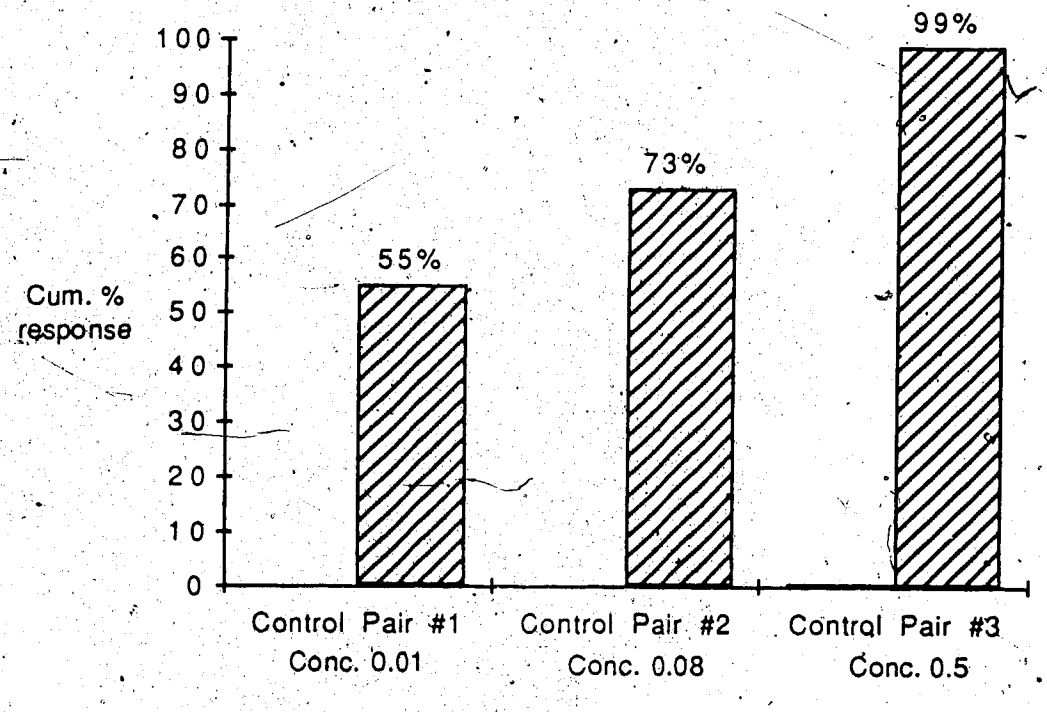

Figure 27. Sensory evaluation, replicate #2, cumulative percent response of exposed or fortified flesh vs control at each of three concentrations.

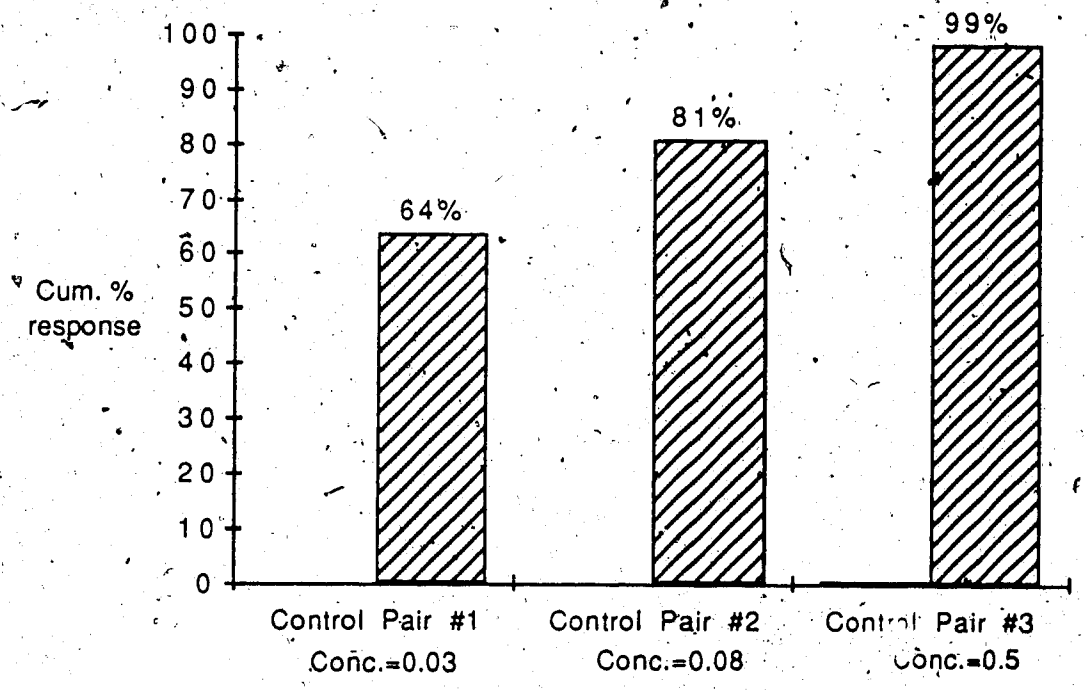

Figure 28. Sensory evaluation, replicate #3, cumulative percent response of exposed or fortified flesh vs control at each of three concentrations.

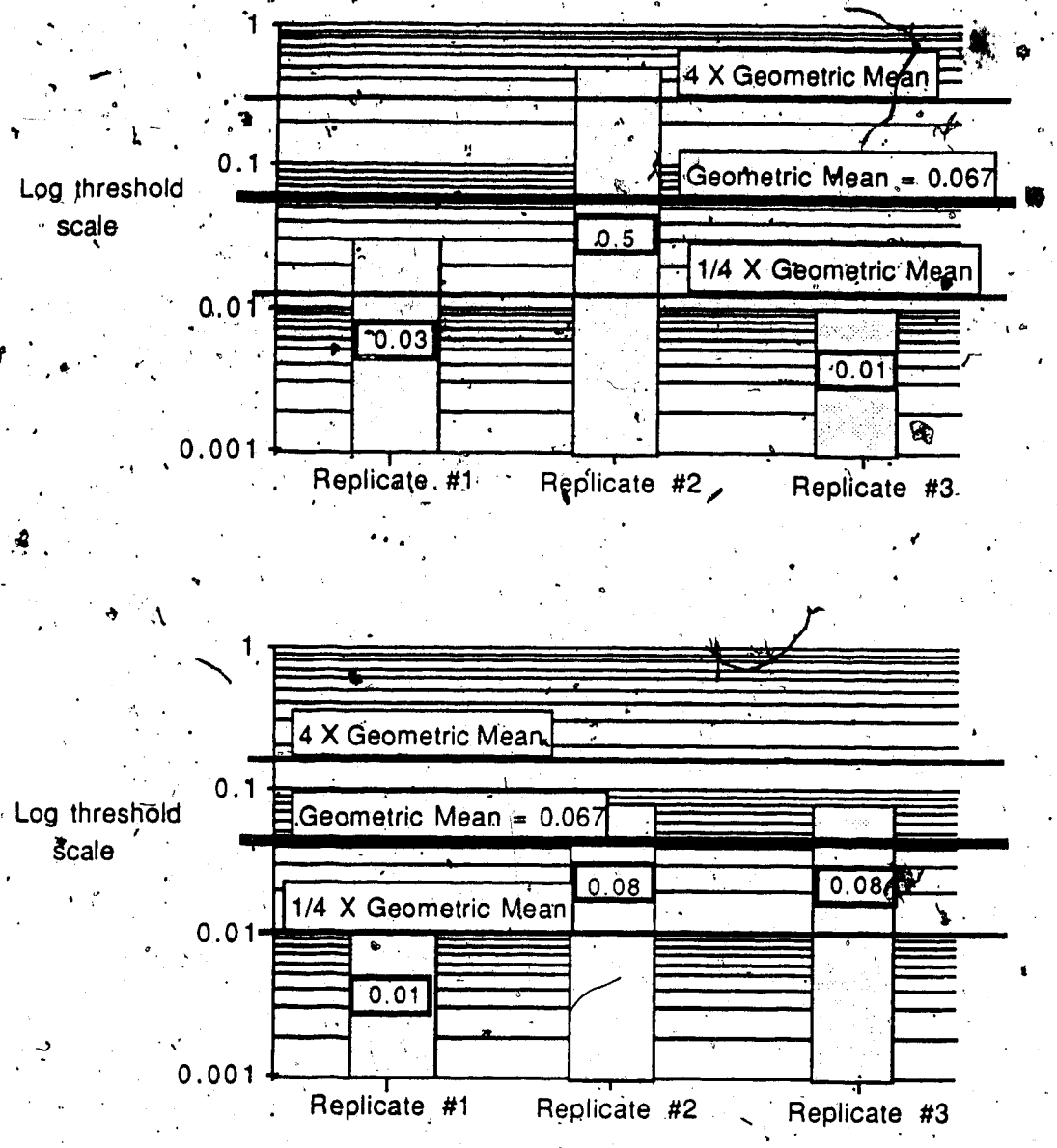

Figure 29. Panelists 6 and 10: plots of individual mean thresholds vs. geometric mean threshold.

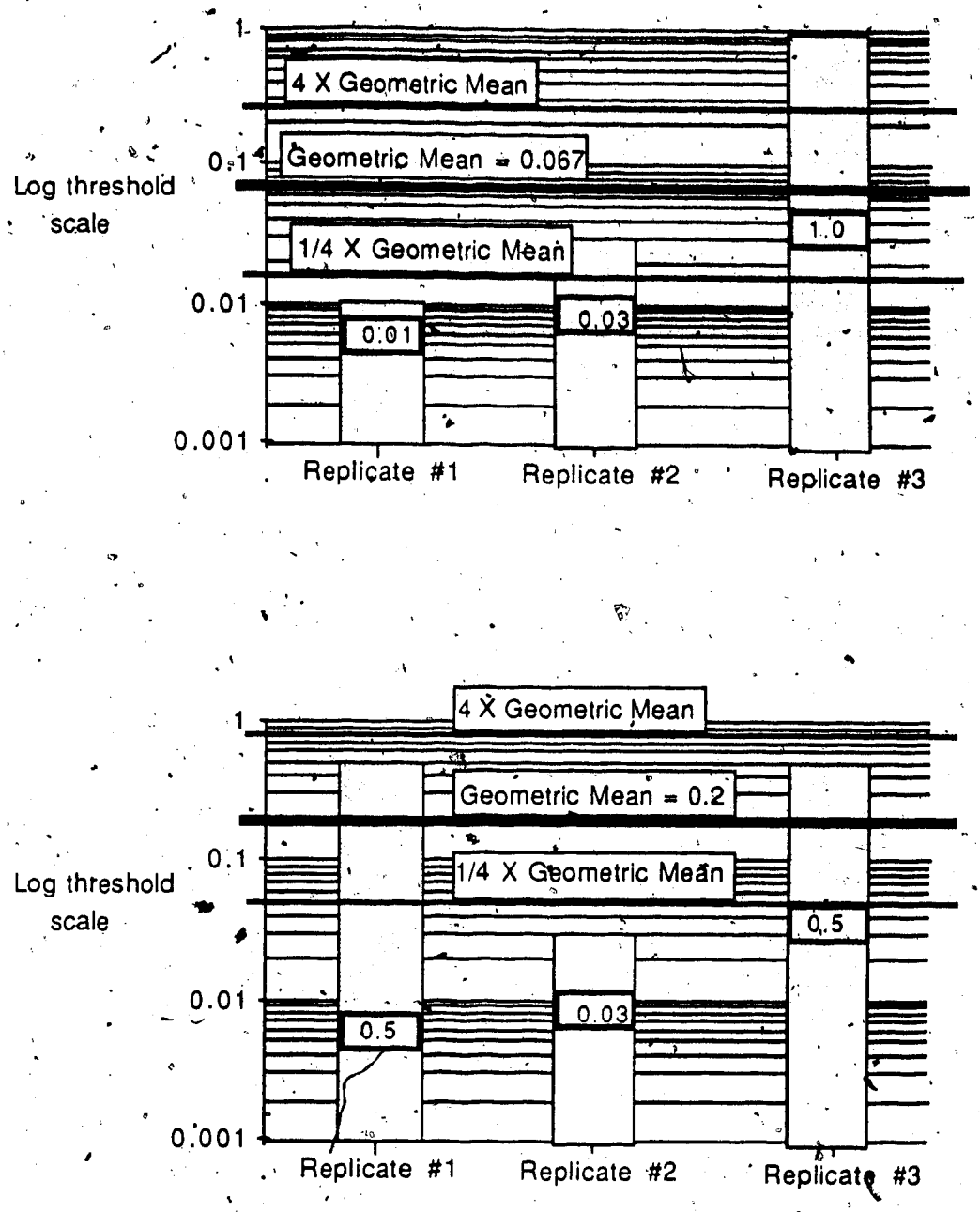

Figure 30. Panelists 1 and 7: plots of individual mean thresholds vs. geometric mean threshold.

4.6.2 Degree of Taint:

Panelists were asked to assign a "degree of taint" on the boxed line provided, to each sample which they choose as being tainted. Panelists were asked to record their response to each sample by placing a mark on the line at a point corresponding to their perceived intensity of the off-flavour in the sample. These values were later converted to numerical scores and are presented in Table 14. Each mean intensity represents a subset of the total number of observations for that treatment, including only individuals who exhibited a threshold odour level below the concentration being judged. Intensities were determined in this manner on the presumption that intensity values assigned for samples which are below each individual's threshold detection level are, by definition not detected and they therefore represent an "incorrect" assignment.

Mean intensities of fortified, exposed and tainted fish are supplied in Table 14. Intensities do not increase proportionally with concentration, as would be expected in such a case. This may be explained by the "saturation effect" (Windholz *et. al*, 1983). At higher concentrations the olfactory senses may be fatigued and fail to perceive increasing concentrations. However, if we do look at the difference in intensities between exposed and spiked samples, we notice an increase in the difference with increasing concentrations. This observation indicates that panelists were more definite in their choice of exposed over fortified at higher concentrations. This observation may be seen more clearly in a graphical representation of the results, provided in Figure 31.

Table 14. Mean intensity scores of fortified and exposed fish; determined from three replicate sensory evaluations using 11 panelists.

Number of Panelists*	Samples	Mean Intensity	Range	
17	E 0.01 ppm	2.3	0-3	E=Exposed fish flesh
5	F 0.01 ppm	0.52	0-7	F=Fortified fish flesh,
21	E 0.03 ppm	1.57	0-6	ppm=parts per million
11	F 0.03 ppm	0.83	0-6	
26	E 0.08 ppm	2.67	0-3	Each mean intensity represents
3	F 0.08 ppm	0.18	0-8	a subset of the total number of observations for that treatment
30	E 0.50 ppm	4.03	0-5	including only individuals who
2	F 0.50 ppm	0.24	0-9	exhibited a threshold odour level
33	E 1.00 ppm	0.82	0-9	below the concentration being judged.
0	F 1.00 ppm			

* There are a total of 33 panelists, based on 11 panelists with 3 replicates.

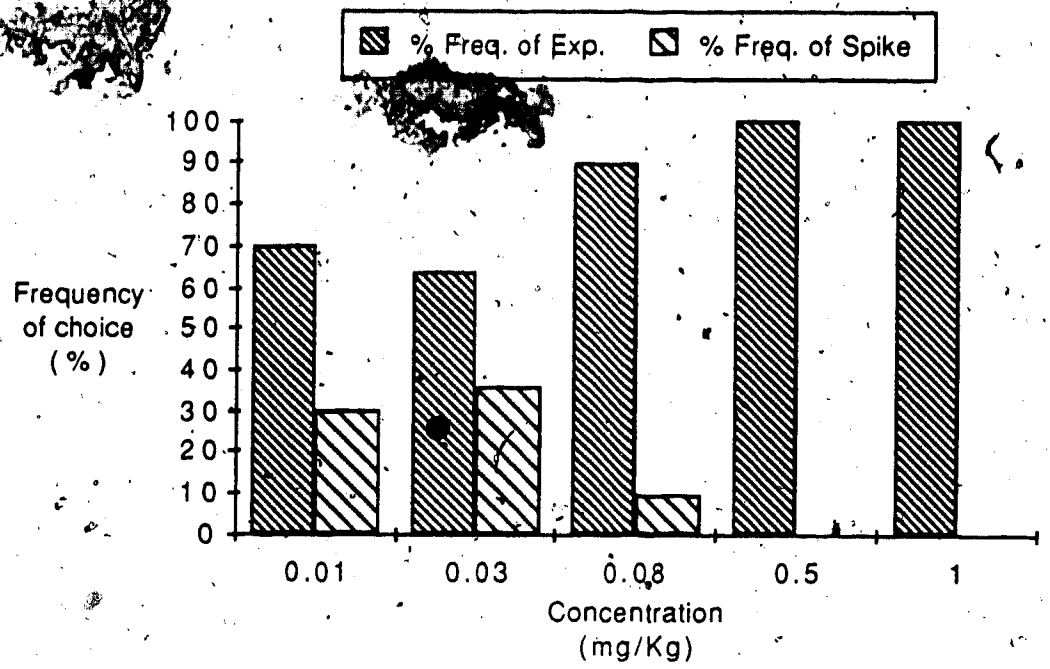

Figure 31. Taste and odour evaluation: average of three replicates.

4.6.3 The Tainted Sample:

The results obtained from the sensory evaluations indicate that the exposed samples are perceived as being more tainted than the fortified samples. This can be seen at a glance in Table 14. The panelists consistently chose the exposed sample as opposed to the fortified, indicated by the large difference in intensities between both the samples. Thus, the spiked approach is conservative as a measure of sensory threshold levels in real life. However the raw data was analyzed for significance of variance between exposed and fortified flesh. From the results of the analysis of variance (ANOVA) presented in Table 15, significant interactions are clearly demonstrated for the taste and odour data. Interactions among concentrations, among taint methods and between concentrations and taint methods were significant at the 1% significance level.

These results can be interpreted as follows:

(1) A significant difference ($F= 17.9$, $P<0.01$) existed between concentration levels and the associated intensity value. The intensity scores increased with increasing concentration.

(2) There was a highly significant difference ($F=265.4$, $P<0.01$) in the selection of taint methods, with exposed flesh being easily favoured over fortified at all concentration levels.

(3) There was a significant interaction ($F=29.8$, $P<0.01$) between the selection of exposed or fortified flesh and the concentration being presented. The frequencies of selections favouring exposed flesh increased with increasing concentration.

In Table 16 an analysis of variance is computed using all perceived intensities, including those below the threshold levels. The results are virtually the same as those

obtained using he perceived intensities above the threshold levels only . The intensity values assigned to the samples by the panelists are provided in Table 17, including those values assigned above the threshold levels only.

Table 15. Analysis of Variance 1: computed perceived intensities above threshold levels only.

	A_1 0.01	A_2 0.03	A_3 0.08	A_4 0.5	A_5 1.0	TOTALS
(Exp.) B_1	Σx=76 Σx^2=412 μ=2.30	Σx=52 Σx^2=222 μ=1.57	Σx=88 Σx^2=376 μ=2.67	Σx=133 Σx^2=675 μ=4.03	Σx=225 Σx^2=1657 μ=6.82	ΣB_1=574 ΣB_1^2=3342 μB_1=3.48
(Fort.) B_2	Σx=17 Σx^2=69 μ=0.52	Σx=27 Σx^2=109 μ=0.82	Σx=6 Σx^2=14 μ=0.18	Σx=8 Σx^2=34 μ=0.24	Σx=0 Σx^2=0 μ=0	ΣB_2=58 ΣB_2^2=226 μB_2=0.35
TOTALS	ΣA_1=93 ΣA_1^2=481 μA_1=1.41	ΣA_2=79 ΣA_2^2=331 μA_2=1.20	ΣA_3=94 ΣA_3^2=390 μA_3=1.42	ΣA_4=141 ΣA_4^2=709 μA_4=2.13	ΣA_4=225 ΣA_4^2=1657 μA_4=3.41	Σx_{tot}=632 Σx_{tot}^2=3568 μ_{tot}=1.92

$SS_{tot} = \Sigma x_{tot}^2 - (\Sigma x_{tot})^2/N_{tot} = 3568-(682)^2/330=3568-1210.37$

$= \underline{2357.63}$

$SS_{bg} = \Sigma[(\Sigma x_g)^2/N_g] - (\Sigma x_{tot})^2/N_{tot} = 8.76+22.1+1.09+1.93+$
$+175.03+81.94+234.7+536.0+1534-1210.37$

$=\underline{1385.18}$

$SS_{conc.} = (\Sigma x_{A1})^2/N_{A1} + (\Sigma x_{A2})^2/N_{A2} + (\Sigma x_{A3})^2/N_{A3} + (\Sigma x_{A4})^2/N_{A4} + (\Sigma x_{A5})^2/N_{A5}$
$(\Sigma x_{tot})^2/N_{tot} = 131.04+94.56+133.88+301.23+767.04-1210.37$

$=\underline{217.37}$

$SS_{taint.} = (\Sigma x_{B1})^2/N_{B1} + (\Sigma x_{B2})^2/N_{B2} - (\Sigma x_{tot})^2/N_{tot} = 1996.82+20.39-1210.37$

$=\underline{806.83}$

$SS_{AB} = 33[(0.4489)+(1.4161)+ (0.0961)+(0.1156)+(3.4225)+(0.4624)+(1.4161)$
$+(0.1089)+ (0.1024)+(3.3856)$

$=\underline{362.16}$

$SS_{wg} = \Sigma[\Sigma x_g^2 - (\Sigma x_g)^2/N_g] = 60.24+86.9+12.91+32.07+236.97+140.06+141.3$
$+139.0+123.0$

$=\underline{972.45}$

ANOVA: Summary table for taste-test

Source	df	SS	MS	F	P
$A_{conc.}$	4	217.37	54.34	17.88	<.01
$B_{taint.}$	1	806.83	806.83	265.40	<.01
AXB	4	362	90.54	29.78	<.01
Within groups	320	972.	3.04		
Total	329	2357.63			

Conclusions of Summary table:

(1) Panelists detected differences in taint between concentrations (increasing).
(2) Panelists detected a taint difference between exposed and fortified flesh.
(3) Panelists detected a greater taint in exposed vs spiked in all cases.

Assuming consistant variability between replicates and judges.

Table 16. Analysis of Variance 2: computed using all perceived intensities, including those below the threshold levels.

	A_1 0.01	A_2 0.03	A_3 0.08	A_4 0.5	A_5 1.0	TOTALS
(Exp.) B_1	Σx=104	Σx=74	Σx=97	Σx=137	Σx=225	ΣB_1=637
	Σx^2=540	Σx^2=314	Σx^2=405	Σx^2=691	Σx^2=1657	ΣB_1^2=3607
	μ=3.15	μ=2.24	μ=2.94	μ=4.15	μ=6.82	μB_1=3.8
(Fort.) B_2	Σx=23	Σx=30	Σx=7	Σx=8	Σx=0	ΣB_2=68
	Σx^2=87	Σx^2=118	Σx^2=15	Σx^2=34	Σx^2=0	ΣB_2^2=254
	μ=0.70	μ=0.91	μ=0.21	μ=0.24	μ=0	μB_2=0.41
TOTALS	ΣA_1=127	ΣA_2=104	ΣA_3=104	ΣA_4=145	ΣA_4=225	Σx_{tot}=705
	ΣA_1^2=627	ΣA_2^2=432	ΣA_3^2=420	ΣA_4^2=725	ΣA_4^2=1657	Σx_{tot}^2=3861
	μA_1=1.92	μA_2=1.38	μA_3=1.58	μA_4=2.20	μA_4=3.41	μ_{tot}=2.14

$SS_{tot} = \Sigma x_{tot}^2 - (\Sigma x_{tot})^2/N_{tot} = 3861-(705)^2/330 = 3861-1506.14$

$= \underline{2354.9}$

$SS_{bg} = \Sigma[(\Sigma x_g)^2/N_g] - (\Sigma x_{tot})^2/N_{tot} = 327.8+165.9+285.1+586.6+1534.1+1608+$
$+273+1.48+1.94+0-1506.14$

$= \underline{1422.1}$

$SS_{conc.} = (\Sigma x_{A1})^2/N_{A1} + (\Sigma x_{A2})^2/N_{A2} + (\Sigma x_{A3})^2/N_{A3} + (\Sigma x_{A4})^2/N_{A4} + (\Sigma x_{A5})^2/N_{A5}$
$(\Sigma x_{tot})^2/N_{tot} = 244.38+163.88+318.56+767.04-1506.14$

$= \underline{151.6}$

$SS_{taint.} = (\Sigma x_{B1})^2/N_{B1} + (\Sigma x_{B2})^2/N_{B2} - (\Sigma x_{tot})^2/N_{tot} = 2459.21+28.02-1506.14$

$= \underline{981.1}$

$SS_{AB} = 33[(0.24)+(1.12)+ (0.13)+(0.05)+(2.86)+(0.26)+(1.12)$
$+(0.13)+ (0.05)+(2.83)$

$= \underline{290.1}$

$SS_{wg} = \Sigma[\Sigma x_g^2 - (\Sigma x_g)^2/N_g] = 212.2+148.1+119.9+122.4+122.9+71+90.7$
$+13.5+32.06$

$= \underline{932.8}$

ANOVA: Summary table for taste-test

Source	df	SS	MS	F	P
$A_{conc.}$	4	151.6	37.9	13.07	<.01
$B_{taint.}$	1	981.1	981.1	338.31	<.01
AXB	4	290.1	72.5	25.0	<.01
Within groups	320	932.8	2.90		
Total	329	2355.6			

Conclusions of Summary table: As in Table 15.

(1) Panelists detected differences in taint between concentrations (increasing).
(2) Panelists detected a taint difference between exposed and fortified flesh.
(3) Panelists detected a greater taint in exposed vs spiked in all cases.

Assuming consistant variability between replicates and panelists.

Table 17. Intensity values assigned to samples including percieved intensities above the threshold levels only.

Judge #	Rep. #	{Concentrations (mg/kg):Exposed (E) vs Fortified (F)} .01E	.01F	.03E	.03F	.08E	.08F	.5E	.5F	1.0E	1.0F
1	1	0	4	0	6	2	0	4	0	5	0	0
	2	0	0	0	1	0	1	2	0	1	0	2
	3	0	0	0	0	0	0	0	0	8	0	0
2	1	7	0	0	1	6	0	9	0	5	0	0
	2	7	0	4	0	2	0	7	0	9	0	0
	3	3	0	3	0	4	0	0	5	7	0	0
3	1	7	0	3	0	3	0	5	0	7	0	0
	2	0	0	0	0	3	0	0	3	7	0	0
	3	0	3	0	4	4	0	4	0	6	0	0
4	1	5	0	4	0	4	0	4	0	9	0	0
	2	7	0	0	1	1	0	5	0	4	0	0
	3	6	0	2	0	0	3	4	0	4	0	0
5	1	4	0	0	1	1	0	3	0	4	0	0
	2	2	0	0	1	3	0	3	0	3	0	0
	3	2	0	0	1	2	0	4	0	7	0	0
6	1	0	6	0	5	2	0	7	0	8	0	0
	2	0	0	0	0	0	2	3	0	9	0	0
	3	0	0	0	0	3	0	7	0	8	0	3
7	1	0	0	0	0	0	0	6	0	8	0	0
	2	0	0	2	0	7	0	3	0	8	0	2
	3	0	0	0	0	0	0	2	0	5	0	5
8	1	0	2	3	0	2	0	4	0	9	0	0
	2	2	0	6	0	2	0	5	0	9	0	0
	3	0	2	0	5	5	0	4	0	8	0	0
9	1	7	0	4	0	5	0	4	0	6	0	0
	2	3	0	0	1	4	0	5	0	8	0	0
	3	5	0	4	0	4	0	4	0	8	0	0
10	1	0	0	6	0	3	0	6	0	7	0	5
	2	0	0	0	0	0	0	1	0	7	0	0
	3	1	0	5	0	3	0	3	0	7	0	0
11	1	5	0	5	0	8	0	6	0	8	0	5
	2	3	0	1	0	4	0	5	0	8	0	0
	3	0	0	0	0	1	0	4	0	8	0	1

5. Discussion

5.1 Methodology:

The simulation of environmental exposure is perhaps the most difficult parameter to incorporate in laboratory tests. However, the use of this particular laboratory study, as opposed to a field study, has several inherent advantages: both genetically and physiologically homogenous material may be chosen; the physiological condition of animals before exposures can be controlled in respect to their nutritional and thermal history; the exact time and duration of the exposure may be known and the chemical concentrations can be closely monitored.

Complete sample homogenization is essential when accurately quantitating xenobiotics in biological samples, especially fish, where the fat is heterogenously distributed. Benville and Tindle (1970) found the dry ice procedure kept the frozen tissue brittle and vulnerable to fragmentation. In this study the dry-ice procedure was found to be effective and efficient, yielding an homogenous sample in the minimum of time.

Solvent extraction was a necessary step in the recovery of the contaminant from the fish tissue. It was found that 280 mL of solvent (methylene chloride) was needed to extract a 20 g sample of fish, mixed with 80 g of anhydrous sodium sulphate. A minimum of 4 h soxhlet extraction was required to achieve >90% recovery. Less time than this yielded poor recovery. In this particular study, a minimum of 5 h soxhlet extraction was observed.

Stalling *et al.* (1972) reported greater than 95% recovery using gel permeation chromatography. Recovery of benzothiophene was 97 +/- 2% and that of

dibenzothiophene was 95 +/- 2%. Rainbow trout have a high lipid content (7-10%) and as such are difficult to clean. However, gel permeation chromatography was found to effectively remove the lipid content of the tissue extract. G.P.C. is unique in that separations are based primarily on differences in molecular size and since molecular weight of most lipids are between 600 and 1500 and those of benzothiophene and dibenzothiophene are between 130 and 200, G.P.C. can effectively separate the lipids from the xenobiotics.

Stalling *et al.* (1972) found bio-beads to be superior to Sephadex in gel chromatography. Since clean-up of the fish extract was essential in our analysis, bio-beads SX3 were chosen in preference to Sephadex. Additional clean-up with florisil is recommended for samples in which whole body PAH residues are less than 0.1 µg/g (Stalling *et al.*, 1972) and since we were unsure as to the quantity of xenobiotics in the fish tissue, florisil cleanup was chosen as an added precaution. The results demonstrate that whole body concentrations greatly exceeded this value, however.

5.2 Accumulation Studies:

There are at least two paths by which fish can take up hydrocarbon (Lee *et al*, 1972 and 1976; Varanasi *et. al*, 1979; Roubal *et. al*, 1977 and Sanborn and Malins, 1977). The first path is with the food, where hydrocarbon entrance is via the gut. The second path is dealt with in this report, namely, uptake of hydrocarbon dissolved in the water, where entrance is through the gills. Varanasi *et al.* (1978) reported a third path of uptake, the skin and demonstrated that the skin of rainbow trout is actively involved in the uptake and discharge of hydrocarbons. In general, however, the literature suggests that the gills are the primary route of uptake.

Lee, *et al.* (1972) suggest that the gills have a micellar layer which adsorbs the hydrocarbons and then passes them on to other tissues. Stein, *et al.* (1984) concur with the theory that the gills are a significant non-dietary route of uptake of hydrocarbons, but suggest that they are not a major route of excretion. McKim and Heath (1983) have shown that 76% of a hydrocarbon in inspired water is adsorbed in one pass, by the gills of two trout species and that negligible amounts are excreted via the gills.

Once absorbed through the gills, the hydrocarbons are then distributed into the blood system and transported via the arterial system to the tissues and organs throughout the body. Most literature reports that the hydrocarbons then build up in the liver, where metabolism first occurs (Lee, *et al.*, 1972; Varanasi and Gmur, 1980 & 1981; Stein *et al.*, 1984 and Thomas and Rice, 1982) . In studies with naphthalene, it has been shown that the majority of this compound taken up by the fish is metabolized in the liver and excreted into the bile. There appears to have been no such studies with benzothiophene and dibenzothiophene. In the absence of any other information, the documented route for the hydrocarbons should be considered possible routes for the two PASH compounds.

The rapid uptake of benzothiophene and dibenzothiophene by rainbow trout was similar to results obtained using naphthalene and phenanthrene as hydrocarbons of interest (Eastmond *et. al*,1984). Both PASH were readily taken up and bioaccumulated in the muscle and liver of rainbow trout. As compared to dibenzothiophene, benzothiophene was taken up more rapidly; accumulated to higher levels in all tissues studied; stored in the liver to a much greater extent as parent compound; and was eliminated more rapidly from muscle and liver tissue.

There was no accumulation of dibenzothiophene in the bile studied. Absence from the bile might indicate rapid metabolism by the mixed-function oxidase (MFO) enzyme

system, as indicated by Stegeman (1981). However, there was no evidence of conjugated or non-conjugated metabolites in the bile studied. In the case of benzothiophene, the liver was found to have the highest concentration , whereas muscle exhibits the highest concentration in the case of dibenzothiophene.

5.3 Elimination studies:

There are two routes of excretion presented in the literature; renal and bilary, with bile appearing to be the major route. Previous studies (Varanasi *et al*, 1982a & 1982b) have indicated that both parent compound and metabolites are deposited in the bile juices. In the case of benzothiophene (no depuration) parent compound was found in the bile juices, but, there was no evidence of parent compound in bile of fish exposed to dibenzothiophene.

Cravedi and Tulliez (1982), suggested that starvation has an adverse effect on rainbow trout for depuration of hydrocarbons. They noticed that the rate of mobilization of stored hydrocarbons by animals fed an energy-restricted diet was less than that of those receiving control diet. They concluded that in aquatic organisms, mobilization of foreign compounds occurred very slowly, especially during starvation. This might be partly due to a slow lipid utilization resulting from a low metabolic rate during starvation. Because of these observations, fish used in depuration studies in this experiment were fed for the duration. Although the fish were reluctant to eat the food in the first 8 h of being in clean water, they commenced eating soon thereafter. All gall bladders expirated for analysis were full, indicating that digestion and mobilization was in process.

The reason why benzothiophene is eliminated from the tissues to a much greater extent than dibenzothiophene is unclear. Possibly as Lee, *et al.* (1972) suggested in the

case of benzo(a)pyrene, dibenzothiophene causes a diminution of the metabolic rate, so that less hydrocarbon is processed and eliminated. This may also explain why less dibenzothiophene is taken up by the fish as compared to benzothiophene. When evaluating the persistence of chemicals in fish, we are instructed by Lech and Bend (1980) to consider that multiple mechanisms of elimination may exist and while metabolism is important for certain compounds, alternate mechanisms may predominate for others. In studies with rainbow trout they found that certain hydrocarbons are metabolized very little, if at all, but can be eliminated gradually over a period of time through the egg mass in the case of the female and the sperm in the case of the male. It may be expected therefore, that such alternate mechanisms are important for compounds that are metabolized slowly *in vivo*. Such may be the situation with dibenzothiophene.

Dibenzothiophene is more persistent than benzothiophene, clearly indicated by the high levels remaining in the liver after depuration. Benzothiophene, on the other hand, decreased by as much as ten fold in depurated livers. Obviously the fish have less trouble eliminating benzothiophene from the tissues, than they do dibenzothiophene.

5.4 Metabolism Studies:

Vertebrates, including fish are able to metabolize various types of hydrocarbons, as outlined by: Diamond and Clark, (1970); Clark and Diamond (1971); Lee *et al.*, (1972); Varanasi and Gmur (1981); Varanasi, *et al.* (1982b); Krahn and Malins (1982) and Krahn *et al.*, (1984). There are numerous papers reporting the metabolism of hydrocarbons such as naphthalene , however, there have been no reports on the metabolism sulphur hydrocarbons such as benzothiophene and dibenzothiophene.

Polycyclic aromatic hydrocarbons are metabolized in the liver by a small number of reactions mediated by enzymes (Kappas and Alvares, 1975): oxidations, reductions, hyrolysis and conjugations. It is known that the introduction into fish of lipid soluble hydrocarbon causes activation of these various enzyme systems (Lee *et al.*, 1972; Sims, 1967). Their essential effect is to convert lipophilic compounds like benzothiophene and dibenzothiophene into hydrophilic ones. These hydrophilic compounds are more easily removed from the blood by the kidneys and excreted.

Initial steps in the bioconversion of aromatic hydrocarbons take place in the endoplasmic reticulum of the cell. The enzymes, aryl hydrocarbon hydroxylases (AHH) operate in unison with the electron transport system of the cell (i.e. via cytochrome P-450) (Roubal, *et al.*, 1978; Malins, *et al.*, 1979, Krahn *et al.*, 1981). Oxygen from the electron transport system combines with the aromatic nucleus to form an epoxide which is subsequently converted to compounds such as mono- and dihydric alcohols (Lech, 1974).

Conjugation is often a second step that comes after metabolism by oxidation, reduction or hydrolysis. Conjugations with natural constituents like glucuronic acid occurs in the presence of the appropriate enzyme. Conjugating enzymes such as glutathione-S-epoxide mediate the additional secondary conversions to conjugate derivatives, which increase the hydrophilic nature of the initial oxidation products. These conjugated derivatives include mercapturic acids, glucuronides, sulphates and glycosides. In my research, the enzyme glucuronidase was provided to remove a possible conjugation product such as glucuronide acid and thus identify the remaining compound, an alcohol, more easily.

Gruger, *et al.* (1977) has previously shown that the AHH of certain salmonids are induced by exposure of the fish to aromatic hydrocarbons, so it is possible that both

benzothiophene and dibenzothiophene induce AHH activity in rainbow trout. The comparatively small amount of benzothiophene found in the liver of depurated fish indicates that the chemical was mobilized in some manner. Although no metabolites were found, it is suspected that benzothiophene was indeed converted to metabolites. A graphical representation of one possible pathway for the metabolism of benzothiophene is illustrated in Figure 21. In this scheme, the benzothiophene is converted initially to the epoxide via the cytochrome P-450 and converted to the monohydric alcohol, followed by an additional reaction of the compound: glucuronic acid is incorporated into the molecule. As such, the metabolite can be more easily discharged through normal renal routes. However, as explained earlier, when the bile extract was enzymatically hydrolyzed with glucuronidase solution, the expected product was not found (i.e. an alcohol). We know that these suspected metabolites are not glucuronides, we also know that they are not phenols, as acetylation of the phenolic compound would have yielded a compound easy to identify with the G.C.

Dibenzothiophene, being more complex, is not readily eliminated; in fact, it accumulates in the liver. It has been demonstrated by Varanasi, *et al.* (1982a) that the major route of excretion of high molecular weight PAH's is via bile in fish. However, in the present study with dibenzothiophene, neither parent compound nor metabolites (conjugated or non-conjugated) were found in the bile and the concentration of dibenzothiophene in the liver did not greatly decrease following depuration. This would seem to indicate that the fish have more difficulty mobilizing dibenzothiophene than they do benzothiophene. This does not automatically lead to the conclusion that rainbow trout cannot metabolize dibenzothiophene. Rather, that it is more persistent. It is possible that there were unidentified metabolites present in the bile. As was the case with benzothiophene, we are assured that these possible metabolites are not glucuronides.

5.6 PASH as petroleum markers:

The present experiment demonstrated that organic sulphur compounds rapidly transfer from the water medium to fish. Since polycyclic aromatic sulphur compounds are contained in crude oil, bitumen and to a lesser extent in refined petroleum products, they may be useful as a marker of oil pollution. Dibenzothiophene, being the more persistent of the two compounds studied would appear to be more suitable as a petroleum marker. The fish can easily accumulate this particular PASH, but have difficulty in its mobilization and elimination.

Previously, researchers have attempted to select compounds contained in crude oil as a marker of oil pollution in fish (Ogata and Miyake, 1973, 1978, Ogata and Ogura, 1976, Krahn *et al.*, 1984 and Oikari and Kunnamo-Ojala, 1987) but no specific compounds were chosen. Ogata and Miyake (1973) suggested that aromatic sulphur compounds be chosen. Although, dibenzothiophene is recommended as a petroleum marker in this particular study, previous studies (Oikari and Kunnamo-Ojala, 1987; Krahn *et al.*, 1984; and Krahn and Malins, 1982) have concluded that metabolites of aromatic hydrocarbons, as opposed to the parent compound, are sensitive and quantitative tools for assessing aquatic contamination.

Krahn *et al.* (1984) suggest that the existence of AHH (aryl hydrocarbon hydroxylase) activity in fish may provide a convenient means of assessing previous exposure to petroleum, or other products containing PAH's. However, not all aromatic hydrocarbons are metabolized in the fish and it is possible that the sulphur hydrocarbon, dibenzothiophene is a similar case. Further research is required to ascertain if this theory is indeed correct. Furthermore, for a better understanding of how the bile patterns of conjugated and non-conjugated metabolites reflect the water quality, more should be known

about bile formation kinetics, not only at constant concentrations, but also at fluctuating ambient concentrations of xenobiotics in the water.

5.7 Tainting potential of benzothiophene:

Heavy pollution and offensive odours may be found in water near petroleum, industrial districts and oil spillage. Baldwin *et al.* (1970) and Ogata and Miyake (1973) identified toluene as a possible main cause of the odour. In this study, benzothiophene was identified as an objectionable odour source. Panelists could detect the compound at concentrations as low as 0.01 mg/kg. From previous studies (Ogata and Miyake, 1973) it was concluded that substances imparting an offensive odour to fish have the ability to infiltrate the fish. Benzothiophene, in this study, certainly meets these two criteria. It has been shown in this experiment, that the compound rapidly enters the fish and does indeed impart an offensive odour.

This appears to be the only research of its kind, to date, which involved exposing fish to a known quantity of benzothiophene and performing a sensory analysis on the resulting flesh. Concentrations were always determined by GC prior to sensory evaluation.

The threshold odour concentration (T.O.C.) for benzothiophene was determined to be 0.01 mg/kg or less, as this was the lowest concentration available to the panelists. Persson (1984) defined estimated threshold concentration (E.T.C.) as the lowest concentration of the compound in water that would lead to impairment of the fish flavour. The T.O.C. is that concentration detectable or recognizable in fish by a group of observers. T.O.C.'s of compounds in the flesh of fish are rarely reported and even less (if any) are

reported for fish actually exposed to a specific compound and analysed by a panel of trained judges, as was the case in this experiment.

In practical situations, it may be important to know whether the flavour of fish will be impaired at water concentrations lower than those detectable by the human olfactory system, i.e. whether E.T.C. is less than T.O.C.. Benzothiophene could fall into this category, as this compound is readily accumulated from low concentrations in the water. Theoretically, it is possible, even probable, given the bioconcentration factors supplied in my research, that the water concentration could fail to impart an offensive odour, but an exposed fish could bioconcentrate the compound and exhibit an obnoxious odour. An important observation from this information, is that the aroma of the water is an inadequate indicator of the fish living in it.

Panelists were asked to choose the most tainted sample from a pair of samples: one exposed to benzothiophene, the other fortified with benzothiophene. Both samples had similar concentrations of benzothiophene. In each of three replicates, panelists consistently chose the exposed flesh over the fortified flesh. This is somewhat baffling, considering the concentrations are so similar. One possible explanation is that during uptake of the compound, fish partially convert benzothiophene to some more obnoxious metabolite, whereas in the fortified sample, there is no manner in which this could occur. However, although metabolism of benzothiophene is suspected, no evidence was found to support this suspicion. Another possible explanation is that the exposed sample is more homogenous that the fortified, given that it was distributed to the muscle via the numerous arteries and arterioles, whereas with the fortified sample, the chemical was mixed in with the aid of a blender.

The latter explanation is somewhat dubious, as the sensory results would seem to indicate that the panelists were consistent in not selecting the fortified fish as being more tainted, whereas a non-homogenous sample should lead to inconsistency rather than consistency. A third possibility may be that of additive or synergistic effects. Mann (1982) and Westman and Hoff (1963, cited in Persson, 1984) found that the presence of other compounds in the water greatly influenced the intensity of off-flavours produced by specific compounds. The fish were exposed in dechlorinated tap water, with residual organics removed by an activated carbon column. Therefore, although the "synergistic effect" is interesting, it is unlikely in this instance.

5.8 Panelists

There were three panelists who had difficulty in identifying the tainted samples. They randomLy choose the control sample over the tainted sample. The extent to which these three panelists were inconsistent is graphically demonstrated in Figures 29 and 30. Although these three panelists did not particularly alter the overall sensory results, they do increase the geometric group mean threshold. Including these three judges, the group mean is 0.04 mg/kg, however, when these three judges are left out of the calculation, the group mean is 0.02 mg/kg.

Because of these differences the results are presented in both cases; using only the eight consistent judges and conversely, using all eleven judges. Because one of the criteria in selecting the judges was that they be consistent in their choice of sample, the geometric mean values excluding the outlyers are most useful. The data show that these particular judges were far from consistent in their choices.

6. Summary and conclusions

A series of exposure/depuration experiments with rainbow trout were performed, using benzothiophene and dibenzothiophene as compounds of interest. The results demonstrate that these polycyclic aromatic sulphur heterocycles (PASH) are readily bioconcentrated from waterborne concentrations. Bioconcentration factors (BCF) were determined and ranged from 10 to 500. Benzothiophene is accumulated more rapidly than dibenzothiophene in all tissues studied, with the greatest concentrations being found in the liver.

Following 65h depuration, concentrations of both chemicals were substantially reduced in all tissues examined. However, dibenzothiophene appeared to be more persistent than benzothiophene, with concentrations ten times those of benzothiophene present in the flesh following depuration. This indicates the fish had more difficulty mobilizing dibenzothiophene.

There was no evidence of metabolism with either of the chemicals examined, however, this does not necessarily mean that there were no metabolites. The methods of analysis employed were those commonly used for PAH. Analytical techniques for the identification of sulphur heterocycles have not been fully researched; further studies in this area are required before conclusive results about metabolism can be obtained.

In order to identify substances imparting an offensive odour to fish, trout were exposed to varying concentrations of benzothiophene (0.01 to 1.0 mg/L) and compared for taint with similar concentrations of fortified flesh. The chemical was found to impart an offensive odour to fish flesh, even at concentrations as low as 0.01 mg/kg. The threshold

odour level was determined to be 0.01 mg/kg., but since this was the lowest concentration presented to the panelists, it may be assumed that the actual threshold level is less than this.

Statistical analysis of the data indicate that exposed flesh is significantly more tainted than fortified flesh. The exposed flesh was easily favoured over the fortified flesh at all concentration levels examined. Considering both samples had similar concentrations (confirmed by GC-FID) there are some possible explanations for this observation. The exposed flesh may have been more homogenous as the chemical was distributed via numerous arteries and arterioles, whereas it was manually added to fortified flesh. However, results indicate that this is not the case. Alternatively, the exposed fish may have converted benzothiophene to some more obnoxious metabolite, whereas this could not have occurred in the fortified flesh.

Although the latter explanation is favoured, there was no evidence of metabolism in the exposed flesh. As mentioned earlier, this may be the fault of the analytical techniques employed rather than the inability of the fish to metabolize the chemical.

7. Recommendations

Oil tainting of aquatic organisms has been a concern for numerous years. Unfortunately the lack of sufficient analytical techniques for identifying and quantifying the compounds has lead to continuing confusion about this topic. Procedures for sensory evaluation are varied and ambiguous. Threshold odour levels of a few relevant contaminants have been reported, however, the methods of determining them have been difficult to reconcile with modern analytical techniques.

High sensitivity of the olfactory system to sulphur containing compounds has been reported in the literature, yet there have been few studies reported on PASH and their ability to taint fish flesh. The techniques reported for PASH analysis are sadly lacking - with no studies reported on the metabolism of such compounds.

In order to properly address the problem of oil tainting, certain deficiencies must be corrected:

* Further research is required on elimination and metabolism of petroleum components. In order to properly assess the oil tainting problem a basic understanding of the biodegradation and elimination of the components is necessary.

* There is a need to determine what levels of exposure to the major compound classes are necessary to cause tainting over short and long-term exposure periods.

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APPENDIX 1

Sensory release and waiver form

RELEASE AND WAIVER

THIS AGREEMENT made the day of , 1986.

BETWEEN:

THE UNIVERSITY OF ALBERTA, (hereinafter called "the University") and CINDY JARDINE, EDEL DROMEY, WENDELL KONING, all of the City of Edmonton, in the Province of Alberta, (hereinafter called "the Investigators"),

OF THE FIRST PART,

- and -

of the City of Edmonton, in the Province of Alberta, (hereinafter referred to as "the Subject"),

OF THE SECOND PART,

WHEREAS the Subject has agreed to participate in an experiment whereby the subject will be making odour and/or taste evaluations on fish tainted by hydrocarbon compounds;

AND WHEREAS the Subject adknowledges that the [redacted]tors have informed the Subject of the nature and conditions of said experiment:

NOW THEREFORE this Agreement witnesseth that in pursuance of the said Agreement, and in consideration of the sum of ONE DOLLAR ($1.00) and other good and valuable consideration, the receipt of which is hereby acknowledged, the Subject, his/her heirs, executors, administrators and assigns, do hereby remise release, acquit and forever discharge, to the extent permitted by law, the University, its staff associated with said experiment, and the Investigators, their and each of their respective heirs, executors, administrators, successors and assigns of and from all manner of action or actions, cause or causes of action, suits, dues, sums of money, claims, demands or damages whatsoever, [redacted]law or in equity, which the Subject has had or now has, or otherwise hereafter can, shall or may have, for or by reason of all KNOWN and UNKNOWN injury, loss or damage which has resulted or may result to the Subject from or in respect of or arising out of any matter or thing whatsoever existing up to the present time, and, more particularly, from or in respect of or arising out of said experiment.

AND the Subject, for the consideration aforesaid, hereby covenants and agrees to notify the Investigators and/or the University of any reactions, allergic or otherwise, which the Subject may experience during or following said experiment.

AND the Subject, for the consideration aforesaid hereby covenants and agrees not to make any claim or to take any proceedings on account of any injury, loss or damage against any

person, firm, or corporation who may claim contribution or indemnity from the University or the Investigators.

IT IS A TERM of this Release that the payment of the said money and the passing of the said consideration as aforesaid is not and is not to be deemed in any way to be an admission of liability on the part of any of the parties hereto.

IN WITNESS WHEREOF I have hereunto set my hand and seal this day of , 1986.

SIGNED, SEALED AND DELIVERED)
in the presence of)

_____ _____

APPENDIX 2

Sensory screening test forms

SCORING OF FISH TAINTING

NAME_____ DATE_____

After evaluating the first pair of samples in the screening test, score the taint of the most tainted sample on the table below. First, record the three-digit code of the most tainted sample, then evaluate the degree of taint of this sample by ticking under the most appropriate category (see "Example" below). Repeat this procedure for each subsequent pair of samples.

Pair No.	Tainted Sample Code	Degree of Taint				
		trace taint	slight taint	moderate taint	pronounced taint	extreme taint
Example	123				✓	
1						
2						
3						
4						
5						
6						
7						

SCREENING TEST FOR TASTE

NAME_____ DATE_____

Eight coded samples are provided. Each of these cups contains weak water solutions of chemicals representing the four basic taste sensations. One or more of these may be a "blank" of distilled water, or may be a duplicate sample.

Rinse your mouth with the water provided and take a bite of cracker before tasting each sample. Taste each sample separately and in the order indicated. For each sample, record under "Taste Description" if the sample is tasteless, or has a sweet, salty, sour or bitter taste.

Sample Code Number	Taste Description
451	
234	
171	
200	
678	
328	
448	
107	

SCREENING TEST FOR ODOUR

NAME _____ DATE _____

Ten coded samples are provided. Each of these test tubes contains a dilute solution of a compound having a typical odour

Sniff each sample separately and in the order indicated. Rinse your mouth with the water provided and take a bite of cracker after smelling each sample. Wait approximately 15 seconds between samples. Record your description of each sample under "Odour Description".

Sample Code Number	Odour Description
951	
752	
326	
903	
452	
621	
587	
321	
834	
787	