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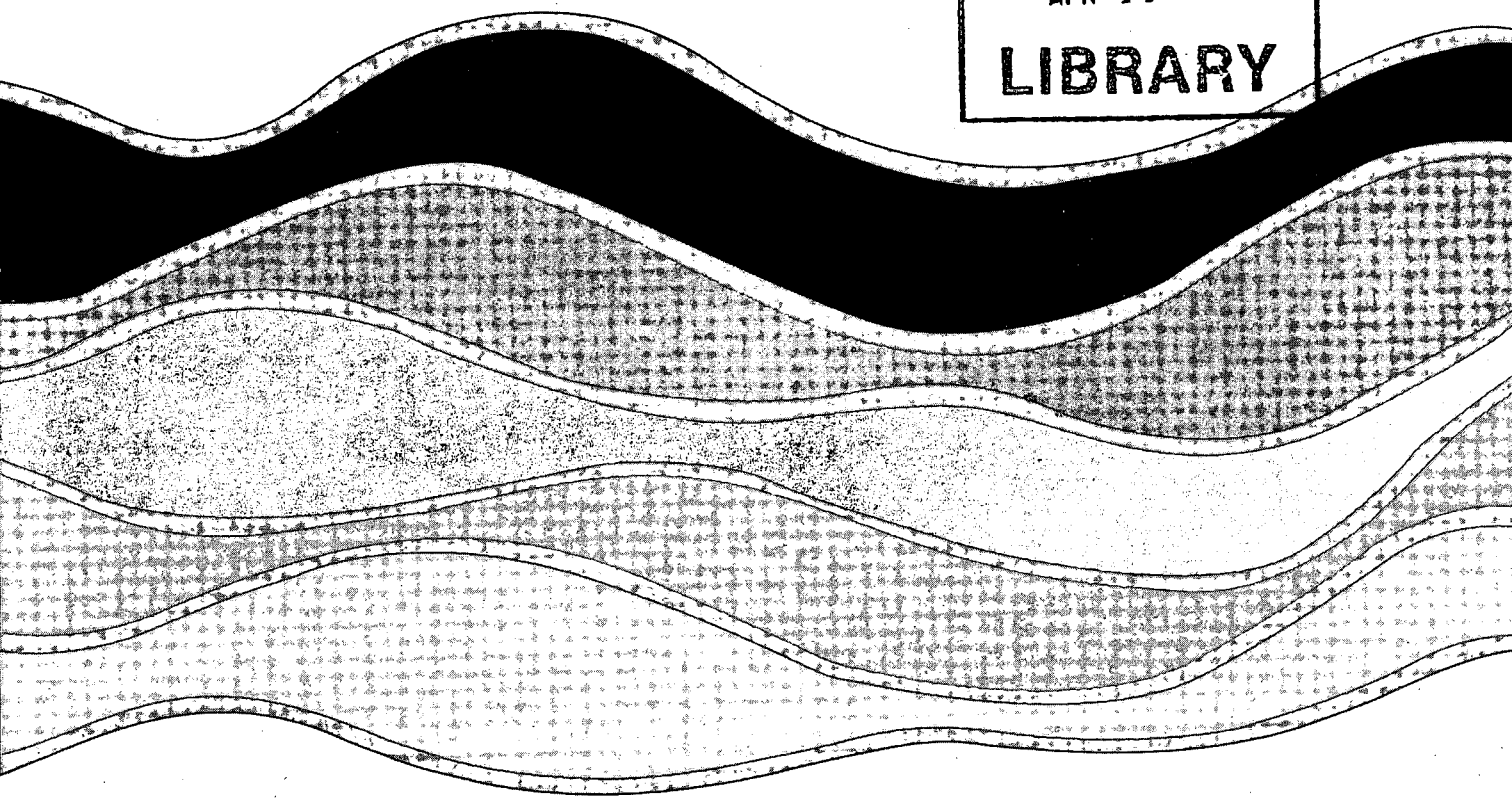

INVESTIGATION OF THE SUBLETHAL EFFECTS OF SOME PETROLEUM REFINERY EFFLUENTS

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NWRI Contribution No. 94-54

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MANAGEMENT PERSPECTIVE

Effluents from petroleum refineries can adversely affect the biota in the receiving ecosystem in several ways. In Canada, current environmental regulations and guidelines have tended to emphasize the control of acute toxicity in petroleum refinery effluents. Petroleum refineries in particular have earned a good reputation for compliance with current regulations and guidelines. There is concern that those effluents may have other subtle, but still deleterious, long-term effects on the aquatic ecosystem. The present study responds to the need for knowledge on the sublethal effects of petroleum refinery effluents. Effluents from two Ontario refineries were non-lethal to rainbow trout and *Daphnia magna*, and two of the effluent samples were non-toxic in the Microtox test. The following toxicity tests detected sublethal effects in some of the effluents: fathead minnow larval assay, *Ceriodaphnia, Selanastrum, Lemna*, seed germination, nematode assay. The SOS-Chromotest detected genotoxicants in one effluent sample. Further research is needed to assess the regulatory and environmental implications of these observations. We also need to know how typical these results are of other Canadian refineries.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Les effluents des raffineries de pétrole peuvent avoir plusieurs types d'effets néfastes sur le biote des écosystèmes récepteurs. Au Canada, les règlements et lignes directrices environnementaux actuels tendent à mettre l'accent sur la toxicité aiguë des effluents des raffineries de pétrole. Par rapport aux autres industries, les raffineries de pétrole se sont mérité une bonne réputation pour ce qui est de la conformité aux lignes directrices et règlements actuels. On se préoccupe du fait que ces effluents peuvent avoir d'autres effets à long terme plus subtils, mais néanmoins délétères, pour l'écosystème aquatique. La présente étude répond au besoin de connaissances concernant les effets sublétaux des effluents des raffineries de pétrole. Les effluents de deux raffineries de l'Ontario étaient non létaux pour la truite arc-en-ciel et *Daphnia magna*, et deux des échantillons d'effluents n'étaient pas toxiques à l'essai Microtox. Les essais de toxicité suivants ont mis en évidence des effets toxiques sublétaux avec certains des effluents, par exemple l'essai avec les larves de tête de boule, l'essai avec *Ceriodaphnia, Selanastrum* et *Lemna*, l'essai de germination de semences et l'essai avec des nématodes. Le SOS-Chromotest a détecté la présence de composés génotoxiques dans un échantillon d'effluent. D'autres recherches sont nécessaires pour évaluer les répercussions réglementaires et environnementales de ces observations. Nous devons également savoir jusqu'à quel point ces résultats sont caractéristiques des effluents d'autres raffineries canadiennes.

ABSTRACT

In Canada, environmental regulations for protection of the biota from the adverse effects of effluents from petroleum refineries have tended to focus on acute toxicity. There is concern those effluents may have other subtle, but still deleterious, long-term effects on aquatic ecosystems. We have used a battery of toxicity tests to assess the acute toxicity, genotoxicity, and chronic toxicity of effluent samples from two Ontario refineries. The test organisms included representatives of the bacterial, algal, plant, cladoceran, and fish communities. The results of our preliminary study indicate that the effluent samples had little acute toxicity to the test organisms. There were indications of some sublethal toxicity to *Ceriodaphnia dubia*, *Panagrellus redivivus*, and *Pimephales promelas*. One of the effluents inhibited the growth of *Selanastrum capricornutum* (IC_{50} of 59.9%) and *Lemna gibba* (IC_{25} of 73.3%) and also caused a 15% reduction in the germination of *Lactuca sativa* seeds. The SOS-Chromotest, a commercially available test that measures the activity of a bacterial DNA repair system, detected genotoxic effects in a single effluent that had been concentrated ten fold. There was no apparent relationship between the chemical composition of the effluents and the observed sublethal effects. Further research is needed to establish whether the observed toxic effects are typical of effluents from Ontario refineries.

RÉSUMÉ

Au Canada, les règlements environnementaux pour la protection du biote des effets nocifs des effluents des raffineries de pétrole tendent à mettre l'accent sur la toxicité aiguë. On se préoccupe aussi de fait que ces effluents peuvent avoir d'autres effets à long terme plus subtils, mais néanmoins délétères pour les écosystèmes aquatiques. Nous avons utilisé une batterie d'essais de toxicité pour évaluer la toxicité aiguë, la génotoxicité et la toxicité chronique d'échantillons d'effluents provenant de deux raffineries de l'Ontario. Parmi les organismes d'essai utilisés, notons des représentants des communautés bactériennes, algales, végétales, des cladocères et des poissons. Les résultats de notre étude préliminaire indiquent que les échantillons d'effluents sont peu toxiques pour les organismes d'essai. On a noté certaines indications de toxicité sublétale pour *Ceriodaphnia dubia, Panagrellus redivivus* et *Pimephales promelas*. L'un des effluents inhibait la croissance de semences de *Selanastrum capricornutum* (CI_{50} de 59,9 %) et de *Lemna gibba* (CI_{25} de 73,3 %), et il entraînait également une réduction de 15 % de la germination de semences de *Lactuca sativa*. L'essai SOS-Chromotest, un essai disponible dans le commerce mesurant l'activité du système de réparation de l'ADN bactérienne, a décelé la présence d'effets génotoxiques pour un seul effluent concentré par un facteur de 10. On n'a pas noté de relations marquées entre la composition chimique des effluents et les effets sublétaux observés. Des recherches supplémentaires sont nécessaires pour déterminer si les effets toxiques observés sont caractéristiques des effluents des raffineries de l'Ontario.

1. Introduction

The effluents from industrial, municipal, and agricultural activities can introduce complex mixtures of chemicals into the aquatic environment. Those effluents can affect the receiving system in various ways. Some add nutrients that can stimulate primary production so causing an ecological imbalance. Others can eliminate or impair components of the biota and thus alter the ecosystem's composition and dynamics. The toxic components of an effluent often act on more than one component of the affected ecosystem: microbes, zooplankton, plants, and fish are each susceptible to the effects of toxic inputs (Mason, 1991).

To-date much attention has been given to the estimation and control of the acute effects of industrial effluents. Tests based on the exposure of fish and other organisms, such as *Daphnia magna*, have been widely used to measure the acute toxicity of effluents and receiving waters (Chapman, 1989; Das and Konar, 1988; EPS, 1974; Feeley and Drummond, 1985; Kszos et al., 1992; Thomas, 1988). That approach, supported in many cases by regulations, has encouraged the use of improved waste treatment processes, which, in turn, have helped to reduce the acute toxicity of effluents from several sources including petroleum refineries.

In Canada petroleum refineries are expected to comply with a set of effluent regulations and guidelines that were enacted into law in 1973 (EPS, 1974). A single biological parameter is defined in the guidelines. It requires that a 24 h fish bioassay (rainbow trout: *Oncorhynchus mykiss*) for acute toxicity be regularly undertaken by the refinery. A 96 h flow through test is to be performed by the responsible government agency at periodic intervals. A refinery effluent should "support at least a 50 % survival rate" of test fish (EPS, 1974).

Long-term exposure to industrial effluents may exert subtle sublethal effects on organisms, communities, and their parent ecosystems. At the organism level those sublethal effects may manifest themselves as decreased reproduction, increased birth defects, increases in various mutagenic end points, tumours, or altered growth and behaviour patterns (Gauthier et al., 1993; Mason, 1991; Metcalfe and Sonstegard, 1984; Rowe et al., 1983). There is a growing awareness that acute toxicity tests, which usually measure lethality to the test organism, do not on their own provide a sufficiently sensitive or accurate estimate of the effects of long-term exposure to effluents (Mason, 1991; Rand and Petrocelli, 1985; Rowe et al., 1983).

For those reasons we initiated a study to assess the sublethal effects of effluents from Canadian petroleum refineries. A battery of tests was used to estimate the lethal and sublethal toxicity of effluent samples from two Ontario refineries; acute, chronic, and subchronic tests were included in the battery. The chemical composition of the effluents was also examined. Our hypothesis was that petroleum refineries discharge sublethal toxicants into freshwater ecosystems.

2. METHODS

2.1. Effluents

Effluent samples were taken from the discharge stream at two Ontario refineries. The identities of the refineries are confidential. Refineries A and B produce, respectively, 11,300 m^3 and 7,300 m^3 of product per day. The effluent treatment systems at both refineries include the following components: an API oil/water separator, dissolved air flotation unit, sour water stripper, biological oxidation unit, and a clarification unit. Dold (1989) has described those processes in a recent review of current practices for the treatment of waste waters from petroleum refineries.

Between January and March of 1993, two samples, two weeks apart, were taken from Refinery A and one from Refinery B. The samples were pumped from 1 m below the surface into plastic lined 20 L buckets. Excess air was excluded from the liner

bags as they were being sealed. Samples of unused plastic liner and liner that had been used to store refinery effluent were extracted with either water or methylene chloride. Examination of the extracts by full scan GC/MS indicated that no components of the refinery effluent had adhered to the liners. Approximately 300 L of effluent were taken on each occasion. Samples for chemical analyses were collected in solvent rinsed glass jars. All toxicity tests were started within 24 h of sample collection.

On return to the laboratory the effluent was mixed in a previously unused polyethylene barrel that had been pre-rinsed with effluent. The mixed effluent was then returned to the 20 L plastic lined buckets and stored in darkness at 4°C until used in the various toxicity tests. Fully characterised natural groundwater was used as the dilution water in the fish and cladoceran tests.

Twenty five mL portions of the effluents were concentrated by rotary evaporation at 45 °C for use in some of the toxicity tests (Dutka et al., 1993; Dutka, 1989). It is not known what effects the concentration process had on the effluents' chemistry. One would expect a significant loss of volatile components. Where necessary the concentrates were diluted with dechlorinated and aerated tap water. The data for the control samples indicate that oxygen depletion did not cause problems in any of the toxicity tests.

2.2. Toxicity tests

The toxicity tests used are listed in Table 1. Because some of the tests were introduced as the study progressed the full battery of tests was not used for each effluent. The various toxicity tests are now described in outline so as to conserve space; detailed descriptions of the tests, including descriptions of the reference toxicants and exposure regimes have been published in the cited references.

Table 1. Toxicity tests used to characterize the effluent samples.

	Effluent Sample		
Test	Refinery A	Refinery A	Refinery B
Rainbow trout	+[1]	+	+
D. magna	+	+	+
Microtox	NOL[2]	+	+
Toxi-Chromotest	NOL	NOL	+
Fathead minnow	+	+	+
C. dubia	+	+	+
S. capricornutum	+	+	+
L. gibba	+	+	+
L. sativa	NOL	+	+
Nematode	NOL	+	+
SOS-Chromotest	NOL	+	+

1: test used.

2: test not on line.

2.2.1. Acute toxicity

The samples were tested for acute toxicity to rainbow trout (96 h exposure) and *Daphnia magna* (48 h exposure). Apart from the use of a static renewal protocol with daily replacement of the effluent in the rainbow trout test, the appropriate Environment Canada test (Environment Canada, 1990a,b) was used for each assay. Ten fish were used in each test chamber for the rainbow trout test; a single chamber was used for each concentration. Three organisms were used in each test chamber for the *D. magna* test; 4 replicate chambers were used for each concentration. The Microtox™ test (Microbics Corp.), which measures toxicity to *Photobacterium phosphoreum*, was also used to detect the presence of acute toxicants in the effluents (Dutka et al., 1989). A Microtox™ test is judged positive if the dose response produced by increasing the proportion of sample in the assay mixture yields an EC_{50} (concentration causing a 50% effect) value. With our procedure the reaction mixture can contain up to 45% (v/v) of effluent. The Toxi-Chromotest (Environmental Bio-detection Products, Brampton, Ontario) is based on the ability of toxicants to inhibit the de novo synthesis of an inducible enzyme (β-galactosidase) in a specially mutated strain of Escherichia coli (Orgenics, 1985).

2.2.2. Chronic and sublethal toxicity

The samples were tested for their chronic or subchronic toxicity to *Pimephales promelas* (fathead minnow), *Ceriodaphnia dubia* (water flea), *Selenastrum capricornutum* (alga), *Lemna gibba* (duckweed), *Lactuca sativa* seeds, and the nematode *Panagrellus redivivus*. For the fathead minnow test we measured the survival and growth of larvae in a static test of 7 days duration (Environment Canada, 1992a). The effluent in the test chambers was renewed daily. Thirty organisms were used in each test chamber for the *P. promelas* test; 3 replicate chambers were used for each concentration. In the *C. dubia* test we measured the reproduction and survival of the organisms in a static test of 7 days duration (Environment Canada, 1992b). The effluent in the test chambers was renewed daily. Ten organisms were used in each test chamber for the *C. dubia* test; 10 replicate chambers were used for each concentration. In the algal (Environment Canada, 1992c) and *Lemna* (ASTM, 1991) tests we monitored the growth of the test organisms under

static conditions for periods of 3 and 7 days respectively. The dilution water for the algal and *Lemna* tests were filtered though 0.45 μM filters. Three replicates were run at each concentration for the *Lemna* test. The *Lemna* test is considered valid if there is a $\geq$ 5 fold increase in the number of fronds in the negative controls. Five replicates were used for each concentration in the algal test. The effects of the effluents on the germination of *Lactuca sativa* seeds and the subsequent elongation of the root and seedling was assessed in a 120 h static exposure (Dutka et al., 1989). The chronic effects of the effluents on the nematode *Panagrellus redivivus* were assessed by monitoring 100 second stage juveniles for a 96 h growth period (Dutka et al., 1989). Lethal effects were estimated from a reduction in the total number of animals in the population. The number of nematodes remaining at the second or third juvenile stages is a measure of sublethal effects. Because growth from the fourth juvenile stage (J4) to the adult requires extensive gene activity, a significant reduction in the number of J4 organisms that mature to the adult stage indicates potential genotoxicity.

2.2.3. Genotoxcity

The SOS-Chromotest kit (Environmental Bio-Detection Products Inc., Brampton, Ontario) was used to measure genotoxicity in the effluents. This test measures the increase in activity of E. coli's SOS DNA repair system after exposure of the organism to genotoxicants (Quillardet et al., 1982; Fish et al., 1985). The samples were tested in the presence and absence of the microsomal fraction (S-9) of aroclor induced rat livers (Moltox, 335 Point Branch Drive, College Park, MD, U.S.A.).

2.2.4. Controls

The procedures used to assure the stability and repeatability of the following toxicity tests have been previously described (Dutka, 1991): Microtox, Toxi-Chromotest, SOS-Chromotest, *Lactuca sativa*, and the *Panagrellus redivivus* test.

2.3. Chemistry

On return to the laboratory, 1 L effluent samples were acidified to pH 2 with 2-3 drops of concentrated HCl, and then extracted three times with dichloromethane (DCM). The combined extract was passed through Na_2SO_4 and then reduced in volume to 1 mL. An atomic emission detector (HP 5921A) coupled to a GC was used to measure the effluents' emission spectra at C (193 nm), S (181 nm), N (174 nm), H (378 nm), P (178 nm), and O (777 nm). A GC-MSD (HP5970) was used in the select ion monitoring (SIM) mode to analyze for priority pollutant PAHs (Table 2) and n-alkanes (Mayer and Nagy, 1992). Scan mode runs (40 to 400 m/e) were also performed to identify additional components in the extracts. An additional 1 L effluent sample was analyzed for phenols (Lee et al., 1989).

Table 2. Priority Pollutant PAHs

#	M.W.	Code	Name
1	128	N	naphthalene
2	152	AY	acenaphthylene
3	154	AE	acenaphthene
4	166	FL	fluorene
5	178	PH	phenanthrene
6	178	AN	anthracene
7	202	F	fluoranthene
8	202	PY	pyrene
9	228	BaA	benzo(a)anthracene
10	228	CH	chrysene
11	252	BbF	benzo(b)fluoranthene
12	252	BkF	benzo(k)fluoranthene
13	252	BaP	benzo(a)pyrene
14	276	IP	indeno(1,2,3-c,d)pyrene
15	278	DA	dibenzo(a,h)anthracene
16	276	BP	benzo(g,h,i)perylene

3. RESULTS and DISCUSSION

3.1. Acute toxicity

The two effluent samples from Refinery A and the single sample from Refinery B were non-lethal (<10 % mortality) to rainbow trout and D. magna. The second sample of Refinery A effluent and the Refinery B sample gave negative results in the Microtox™ test at concentration levels of 1x, 10x, and 25x. The Toxi-Chromotest also failed to reveal acute toxic effects in the Refinery B effluent at concentration levels of 1x, 10x, and 25x. The Toxi-Chromotest was not on-line for the Refinery A samples. The foregoing results suggest that acute toxic effects have been successfully controlled in the effluents of the surveyed refineries.

3.2. Chronic and sublethal toxicity

The results of the fathead minnow, *Ceriodaphnia*, *Selenastrum*, and *Lemna* chronic toxicity tests are summarized in Table 3. The first of the samples from Refinery A affected the growth of the fathead minnow larvae (no effect concentration (NOEC) of 25%) and the survival of *Ceriodaphnia* (NOEC of 50%). The other effluent samples were non-toxic to both organisms under our test regime. Storage of the second effluent sample from Refinery A and the Refinery B effluent for periods of 4 and 8 days respectively did not alter their non-toxic status in those tests. A *D. magna* test for chronic toxicity (7 day exposure) failed to detect any toxicity in the Refinery B effluent. That toxicity test was not used to test the Refinery A effluents.

The results from the algal and *Lemna* toxicity tests for the first Refinery A sample were invalidated because of poor growth in the negative controls, which is unfortunate because of that sample's toxicity to the fathead minnow and *Ceriodaphnia*. Also, for logistical reasons the seed germination and 7 day *D. magna* tests for the detection of chronic toxicity had not yet been introduced into our battery of tests at that time. Sample #2 from Refinery A inhibited the growth of both *Selenastrum*, with an IC_{50} of 59.9% (concentration causing 50% inhibition of growth) and *Lemna*, with an IC_{25} of

73.3%. The second effluent sample from Refinery A also caused a 15% reduction in the germination rate of the *Lactuca sativa* seeds: a borderline result that needs to be confirmed by further experiments. Thus, the *Selenastrum*, *Lemna*, and possibly the seed germination assays each detected chronic toxic effects in the second sample from Refinery A suggesting that components of that effluent were toxic to plant life. Neither the Refinery A effluent, sample #2, nor the Refinery B effluent caused a reduction in either the main root length or seedling length of the germinated Lactuca sativa seeds. The Refinery B effluent was non-toxic in the algal and Lemna tests.

The Refinery A effluent (sample #2) was slightly lethal to nematodes (Table 4). The effect was weak, however, and only became apparent when the effluent was concentrated by a factor of 10 times. A sample is considered positive in the nematode test if the survival or maturation rate is less than 90% of the control rate (Dutka et al., 1989). No such effect was apparent in the Refinery B effluent. Only the nematode and *Ceriodaphnia* tests revealed lethal effects, measured as reduced numbers of survivors, in the effluents.

Table 3. Chronic toxicity of refinery effluent to fathead minnow, *Ceriodaphnia*, *Selenastrum*, and *Lemna*

Sample	Fathead minnow		*Ceriodaphnia*		*Selenastrum*	*Lemna*
	Growth	Survival	Reproduction	Survival	Growth	Growth
Refinery A Sample 1	NOEC[1] 25% LOEC[2] 50%	NOEC 100%	NOEC 100%	NOEC 50%	Invalid[3]	Invalid
Refinery A Sample 2	NOEC 100%	NOEC 100%	NOEC 100%	NOEC 100%	IC_{50}[4] 59.9%	IC_{25} 73.3%
Refinery B	NOEC 100%	NOEC 100%	NOEC 100%	NOEC 100%	NOEC 100%	NOEC 90%

1: NOEC is the highest concentration of effluent that had no observable affect on the test's endpoint.

2: LOEC: the lowest concentration of effluent that had a significant affect on the test's endpoint.

3: Invalid: the test failed because of poor performance by the control organisms, see the text.

4: IC_{50}: the concentration of effluent causing 50% inhibition of growth.

Table 4. Toxicity of refinery effluents in the nematode toxicity test.

Sample	Conc. factor (X)	Survivors (%)[1]	Maturation (%)
Refinery A	10	88	96
Sample 2	25	87	28
Refinery B	10	97	103
	25	92	103

1: Survival is expressed a % of the control organisms.

3.3. Genotoxicity

The SOS-Chromotest is considered positive if the induction factor is 1.25 - 1.3 or higher (Dutka, 1989). By that criterion the second sample of effluent from Refinery A (Table 5) did not contain detectable genotoxicants, and the Refinery B effluent gave positive results when concentrated by factors of 10X and 25X. The positive response in the absence of metabolic activation (minus S-9) indicates the presence of mutagens. The larger induction factors for the S-9 treated samples may reflect the combined action of both mutagens and promutagens, or may simply result from promutagenic activity alone.

A strong putative genotoxic (inhibition of maturation) effect was observed in the nematode toxicity test when the second sample from Refinery A was concentrated by a factor of 25 (Table 4). There was no associated increase in lethality for the sample when the concentration factor was raised from 10X to 25X. That observation indicates the presence of chemicals that can disrupt the nematodes' genetic materials. No such effect was seen for the Refinery B sample - although the SOS-Chromotest had detected genotoxic effects in that sample. Thus, the data from the genotoxicity tests are somewhat contradictory.

Table 5. Genotoxicity of petroleum refinery effluents in the SOS test

Sample	Conc. factor (X)	SOS-Chromotest Induction Factor	
		-S-9	+S-9
Refinery A	1	0.96	1.03
(sample 2)	10	0.88	1.12
Refinery B	1	1	1
	10	1.3	1.4
	25	1.5	1.7

3.4. Concentration effects

Rotary evaporation at 45 °C was used to concentrate the effluent samples by up to 25X for some of the toxicity tests. For the nematode and SOS-Chromotest tests the concentrated sample is diluted by a factor of approximately ten in the reaction mixture. Thus, in those tests a sample that has been concentrated by a factor of ten is actually tested at close to the equivalent strength of the raw effluent. It is likely that the effluents were altered during the concentration process. For example volatile chemicals, if present, could have been lost, and otherwise innocuous chemicals may have been boosted to toxic levels. For that reason the biological and ecological significance of the sublethal effects that were detected in the concentrated effluents are unclear. The effects of the concentration process on the chemistry and toxicity of refinery effluents is being evaluated in the second phase of the study. It is possible that other concentration techniques may prove to be more suited to refinery effluents.

3.5. Chemistry

The oil and grease parameter was not included in the present study because of the well known variability problems with current techniques for the measurement of that parameter (ASTM, 1991a; CPPI, 1990). The levels of individual PAHs (Figure 1) in the effluents were < 50 ng/L (ppb). There were higher levels of naphthalene and phenanthrene in the Refinery A effluent. The Refinery A effluent also contained low levels of benzo(a)pyrene. Many PAHs are converted by liver enzyme systems into metabolites that can be mutagenic, carcinogenic, or cytotoxic (Babich and Borenfreund, 1987; Babich et al., 1988; Pucknat, 1981). It has also been

shown that PAHs can be photodegraded into forms that are acutely toxic to fish (Landrum et al., 1984). All priority pollutant PAHs that were detected in the effluents are included in Figure 1. The results of the MSD scan mode runs are reported elsewhere (Scott et al., 1993). There were no measurable amounts of phenols in the samples (detection limit = 1 ppb). Figure 2 shows that the alkane levels were highest in the Refinery A effluent, and that the alkane distribution profiles differed for the two refineries. The element specific chromatograms (Figure 3) show that organo-sulphur compounds were present in the effluents from both refineries. The elution pattern suggests that the carbon-sulphur compounds in the Refinery A effluent were less volatile and had higher molecular weights than those in the Refinery B sample. The AED chromatograms also show that the levels of some phosphorous compounds were higher in the Refinery B effluent than in the Refinery A samples. There were both qualitative and quantitative differences between the phosphorous profiles for the Refinery A and B effluents; whereas, the differences between the profiles for the two Refinery A samples were mainly quantitative. Further studies are needed to substantiate, and, if necessary, explain those differences.

At this stage it is difficult to relate the data on the chemical composition of the effluents to the observed toxic effects. PAHs would be obvious candidate culprits in an investigation into the identity of possible genotoxicants. Such a study would also have to evaluate the possible effects that the concentration procedures, which were used in the genotoxicity tests, have on the effluents' chemistry. Effluents from oil refineries are also known to include a variety of other toxicants such as heavy metals and ammonia (Dold, 1989; Hallett, 1978; MOE, 1989).

3.6. Comparison with other studies

Rowe et al. (1983a) studied the sublethal effects of effluent from a Canadian oil refinery on rainbow trout. The trout were exposed to the effluent for 44 days under a flow through regime. The growth of the young fish was severely affected at an exposure of 30% treated effluent. The NOEC for the sublethal inhibition of growth was estimated to occur at a concentration of 6% effluent. Effluent from the same refinery had a mean LC_{50} of 76% toward *D. pulex* in a 48h test for acute lethality (Westlake et al., 1983). The 14 day LC_{50} was 6.4% effluent and the EC_{50} for reproductive failure was 3.1%. Reproduction of *D. pulex* was apparently more sensitive than several fish parameters (x2.6) to the refinery effluent. The no effect level was at 0.92% effluent. The authors concluded that the effluent would be diluted to the no effect level at most refinery sites in Canada. The effluents tested in the present study were less toxic to *D. magna* (48 h and 7 days) and *Ceriodaphnia* (7 days), which suggests that the quality of the effluent from Ontario refineries may have improved in the decade since the completion of the Guelph group's study. The comparison is weakened, however, by the use of different, though related, test organisms, and the possibility that the effluent samples are not representative.

Treated effluent from an Indian oil refinery (API separator, clarifier, biological oxidation) was reported to be toxic to *S. capricornutum* at 5% effluent (Gaur and Kumar, 1986). There was a negative correlation between the concentrations of oil and phenol and algal growth. The combined effluent from a petrochemical industrial centre in south Finland, the main component of which was a petroleum refinery, inhibited reproduction of *D. magna* in a 21 day test (EC_{50}: 3%); no acute lethal effects were detected (Nikunen, 1985). The present data suggests that the

sublethal toxicants in effluents from refineries A and B were at a lower level than those from the Finnish and Indian refineries - assuming that the *Ceriodaphnia* and *D. magna* (21 days) tests can be compared.

4. Conclusions

The results of our preliminary study show that the effluent samples had little acute toxicity to the test organisms. There were indications of low level sublethal toxicity to *C. dubia*, *P. redivivus*, and *P. promelas*. The failure to detect consistent toxicity in the tests that were run on more than one effluent suggests that the plastic liners used to store the samples during transportation to the laboratory were not a source of toxicity. The second of two effluent samples from Refinery A inhibited the growth of *Selanastrum* (IC_{50} of 59.9%) and *Lemna* (IC_{25} of 73.3%) and also caused a 15% reduction in the germination of *Lactuca sativa* seeds. The SOS-Chromotest detected genotoxic effects in a single effluent. Based on these preliminary results, the following assays should be considered for inclusion in a more detailed investigation of the sublethal effects of petroleum refinery effluents: fathead minnow, *Ceriodaphnia, Selenastrum, Lemna*, seed germination, SOS-Chromotest, and nematode. It would also be beneficial to study the longer term effects of effluent on young fish. The results prompt several questions: (1) Does the sublethal toxicity of refinery effluents vary with time and between refineries? (2) Should other sensitive sublethal endpoints, such as cytochrome P4501A1 induction, be included in our follow up studies? (3) What are the long-term effects of the refinery effluents on the aquatic ecosystem? We hope to address those questions in future studies.

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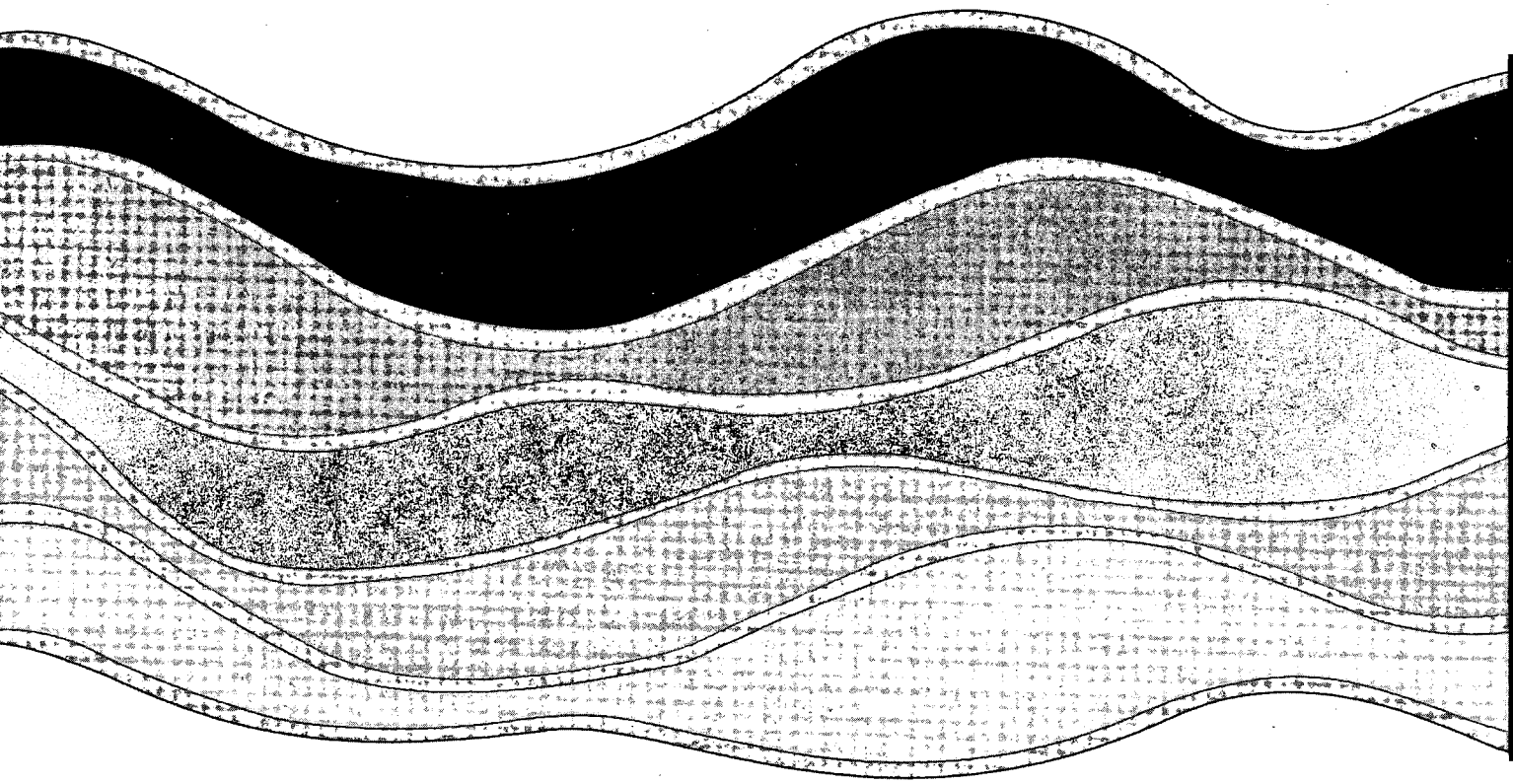

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