THE AQUATIC TOXICITY OF SCRAP AUTOMOBILE TIRES

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THE AQUATIC TOXICITY

OF

SCRAP AUTOMOBILE TIRES

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Tom Millar of the Turkey Point Property Owner's Association collected scrap tires and water samples from an artificial reef in eastern Lake Erie.

EXECUTIVE SUMMARY

The potential for contamination of surface water by automobile scrap tires is a concern because tires have been proposed for use as artificial reefs and floating breakwalls in lakes and rivers. Several laboratory studies have revealed that whole tires or tire pieces placed in a tank of water (static, no flow) release chemical substances lethal to certain aquatic species, especially rainbow trout. The lethality was observed for tires that had not been previously placed in water or exposed to an aquatic environment. One study tested tires that had been previously exposed to an aquatic environment (tires collected from a ten-year-old floating breakwall in Lake Ontario). These tires were placed in a tank of static water, but were found to be nonlethal to trout. Field studies have noted that fish and other aquatic life are attracted to aquatic tire structures soon after construction. Clearly, water flow and other natural processes such as biodegradation must be considered when assessing the potential toxicity of tires in an aquatic environment.

Therefore, the release of chemical substances from tires to water needs to be investigated with tests designed to better simulate field conditions. The purpose of this study was to test scrap tires placed in flowing water, since water flow and dilution largely determine the occurrence of toxicity in natural waters. For the present study, a group of tires was collected from an artificial reef in Lake Erie and from a tire trench site in contact with groundwater. Also collected for testing were two groups of recently-discarded tires that had not had contact with an aquatic environment.

The first objective was to measure the rate of chemical release in flowing water. Each group of tires was placed in a tank of flowing water for several months, and water samples were collected periodically for trout lethality tests. The tests showed that the tires were nonlethal to trout at a minimum water flow rate of 1.5 litres per minute per 600 litre water volume, a flow less than that provided by most Ontario surface waters. Therefore tires in Ontario waters are not expected to cause acute lethality because of sufficient natural dilution. Other environmental processes such as biodegradation, photolysis and particle-binding may also reduce contaminant levels in waters around an aquatic tire structure.

The rate of chemical release decreased during each tire submersion period in the flow-through tests, probably because of a continuous process of leaching. Chemical release also may have been blocked by a bacterial growth that formed on the surface of the submersed tires. The composition of the rubber at the tire surface also may have changed over time in flowing water, possibly affecting chemical release.

The second objective was to compare the toxicity of groups of tires that had had different exposure periods to an aquatic environment. Each group of tires was placed in a tank of static water without flow. After several days, a water sample was collected for a trout test and for chemical analyses. The trout tests showed that tires collected from the artificial reef in Lake Erie were less toxic than scrap tires that had not been previously exposed to an aquatic environment. The chemical analyses detected many more contaminants released by the previously unsubmersed scrap tires than by the reef tires. Previous exposure to an aquatic

environment probably depleted the reef tires of leachable chemicals. There may have been other environmental processes (for example, a change in the composition of the tire rubber) that were responsible for the lower toxicity in static water tanks of the tires collected from the artificial reef compared to the previously unsubmersed scrap tires.

The third objective was to characterize, identify and confirm the toxicant found in static tire water. The toxicant could not be identified, but it was characterized as an organic mixture and aromatic amines were the suspected principal component. The report provides guidance for a limited further investigation to identify some of the principal toxic constituents.

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1.0 INTRODUCTION

The potential for contamination of surface water by automobile scrap tires is a concern because tires have been proposed for use as artificial reefs and floating breakwalls in lakes and rivers. Laboratory studies, reviewed in a previous report (Abernethy, 1994) have revealed that whole tires or tire pieces placed in a tank of water (static, no flow) release chemical substances lethal to certain aquatic species, especially rainbow trout. The lethality was observed for tires that had not been previously placed in water or exposed to an aquatic environment. One study (Day *et al.*, 1993) tested tires that had been previously exposed to an aquatic environment (tires collected from a ten-year-old floating breakwall in Lake Ontario). These tires were placed in a tank of static water, but were nonlethal to trout. Field studies have noted that fish and other aquatic life are attracted to aquatic tire structures soon after construction (Mueller and Liston, 1994; Nelson et al., 1994).

Clearly, water flow and other natural processes such as biodegradation must be considered when assessing the potential toxicity of tires in an aquatic environment. Therefore the release of tire chemicals needs to be investigated with tests designed to better simulate field conditions. The purpose of the present study was to test scrap tires placed in flowing water, since water flow and dilution largely determine the occurrence of toxicity in natural waters. The specific objectives were to:

(1) measure the rate and extent of chemical release from tires placed in flowing water;

(2) compare the acute lethality of tires that had been previously submersed in an aquatic environment for various periods of time; and

(3) characterize, identify and confirm the toxicant found in static tire water.

2.0 METHODS

To investigate the chemical leaching process (objective 1), rainbow trout acute lethality tests were conducted on samples of water in contact with whole tires. The tires were placed in flowing water or in a fixed-volume of static water for a period of time to prepare *flow-through* or *static tire water* respectively. Tire water samples were collected and diluted to different ratios, and trout were exposed to the dilution series following a standard test procedure. The trout tests were conducted on four groups of scrap tires described in section 2.1 (objective 2). To characterize and identify the toxicant (objective 3), a *Toxicity Identification Evaluation* was conducted. Samples of static tire water were subjected to bench-top treatments, and trout tests were conducted on the treated waters. Similarly, *tire crumb* was leached with water, and the water was treated for toxicity tests of two daphnid (small crustaceans) species. The tire crumb was 30 to 40 mesh-size particles of granulated scrap tires, devoid of the fine fibre and steel components normally found in tires. Extensive chemical analyses were conducted on the static tire water and on the tire crumb water.

2.1 Tire water

Whole scrap tires, ranging in weight from 7 to 12 kilograms each, were used to prepare flow-through and static tire water. The tires were from four sources and were tested as separate groups. The tire groups were named and denoted as *A*, *B*, *C* and *D*:

Reef tires (A) Five scrap tires were collected in March 1994 from an artificial reef in Lake Erie, near Turkey Point. Three tires were smaller than average so the total amount of tire material was about equal to four average-sized tires. The reef had been constructed in November, 1992 with about two thousand tires sunk in twenty-three feet of water. An ambient water sample for chemical analyses was collected at the same time as the tires were removed for testing.

Trench tires (B) Four tires were collected in July, 1994 from a covered trench just north of Mount Forest, Ontario. The trench had been excavated around 1991 for an unauthorized tire site, holding about 33,000 tires. The trench was partly-filled with ground water from a fluctuating water table. A surface water sample, for a trout test and chemical analyses, was collected from a connecting trench, suspected of having contact with the tires.

Scrap tires (C) Four tires of a set discarded from an automobile. None had been placed in water or exposed to an aquatic environment before the present study.

Scrap tires (D) Three tires of a set discarded from an automobile. One of the tires had been placed in water for a previous toxicity study, described in Abernethy (1994). In that study, the tire had been placed in a 300-litre water tank on three separate occasions for a total of thirty-six days, and in a 600-litre flow-through tank for twelve days. The other two scrap tires of group D had not been placed in water or exposed to an aquatic environment before the present study.

Each of the four groups of scrap tires was tested more than once. A total of three *flow-through tire waters* and fourteen *static tire waters* were prepared, as specified below. Unless indicated otherwise, the tires were not washed or cleaned, but were tested as received.

2.1.1 Flow-through tire water

Flow-through tire water was prepared in a polycarbonate tank (177 x 66 x 60 cm). A group of four or five tires was submersed in a 600-litre volume of water, and the water flow rate was controlled with a head tank. The water inlet and outlet were at opposite ends of the tank so the water flowed over the tires. A water sample for a trout test was collected near the outlet at various times over the submersion period. The flow rate was adjusted periodically, and the tire water from the previous flow rate was flushed out before further water samples were collected. A second tank without tires was the experimental control. A flow-through experiment with flow rates adjusted over time was conducted for each of three groups of tires (see Table 1).

Table 1. *Flow-through tire water preparation conditions.*

tire group[1]	submersion period (days)	range of flow rates (L/min)	no. water samples collected
A reef tires	84	0.5-2.0	13
B trench tires	78	0.16-1.0	18
C scrap tires	42	0.5-1.5	6

[1] tires described in section 2.1.

2.1.2 Static tire water

Static tire water was prepared by placing a number of tires in a fixed volume of water for a submersion period, then a water sample was collected for a trout test. Table 2 gives the specifications for each of fourteen batches of static tire water, identified as numbers *4* to *17*. The tires of groups A and D were used to make several consecutive batches of tire water. Sometimes, a water sample was collected but the tire submersion was continued at a reduced water volume. A batch number followed by *a*, *b* or *c* refers to a tire water volume reduced by sampling. During the tire submersion period, the water was aerated using a glass air stone and an aquarium air pump. A second water tank without tires was the experimental control.

Water temperatures ranged from 14 to 18 °C during the submersion periods. The static tire waters and control waters were similar in pH (8.0 to 8.4), dissolved oxygen concentration (8 to 10 mg/L) and specific conductivity (324 to 362 µmhos/cm). The water in contact with tires became a pale green-yellow colour and had a *fishy*, ammonia-like odour. The colour may indicate oxidation products of aromatic amines and/or phenols, and the odour is a characteristic of aliphatic amines. The tire surfaces were coated with a clear slimy material after each submersion period, probably due to bacterial growth. The control water was clear and odourless.

2.2 Toxicity Identification Evaluation (TIE)

Samples of static tire water were collected for trout tests, chemical analyses and for bench-top treatments designed to characterize the physical-chemical properties of the toxicant. The effectiveness of each treatment was assessed by comparing the toxicity of a treated sample to the toxicity of an unaltered *baseline* sample. Samples of dilution water were subjected to the same treatments and were tested to check for experimental artifacts, such as potential toxicity caused by an extraction solvent. All the samples of a tire water were not tested simultaneously. Some were stored for further treatments and testing. Stored samples were held as 20-litre aliquots in filled, sealed plastic-lined buckets at 15 °C in the dark. Unless indicated, a sample of tire water was collected, held, treated and tested all in the same 20-litre bucket.

Table 2. *Static tire water preparation conditions.*

tire water batch	submersion period (days)	no. tires	water volume (litres)	tire group[1]
4	12	1[2]	300	D scrap tires
5a	16	3	300	
5b	26	3	220	
6a	8	3	300	
6b	13	3	280	
7	13	1[3]	300	A reef tires
8	13	1	300	
9	13	3	300	
10	15	3	300	
11	12	3	300	
12	11	3	300	
13a	3	3	300	
13b	6	3	280	
13c	13	3	260	
14	11	3	300	
15	3	5	600	
16a	2	4	600	C scrap tires
16b	3	4	580	
16c	15	4	510	
17a	1	4	600	B trench tires
17b	4	4	580	

[1] The tires are described in section 2.1; [2] This tire had been used for a previous study, described in section 2.1; [3] This tire was washed and scrubbed with water to remove the mud and zebra mussels found on all the tires from Lake Erie. All other tires were tested as received, without cleaning or washing.

Samples of static tire water were subjected to the following treatments (the appendices give further details of the treatment methods):

Aeration - Aerated for 24 hours.

Pre-tested - Ten fish were exposed for a 24-hour test, the fish were removed and ten more fish were added for a second test of the same sample.

Steam distillation - Four litres were boiled to reduce the volume to three litres. Distilled water was used to reconstitute the original volume.

Sulphur binding - A 24-hour nonlethal concentration (560 µg/L) of mercuric chloride was added to bind organo-sulphur compounds.

Carbon sorption - Activated charcoal was added to tire water one day before the test fish.

Carbon extraction - Activated charcoal was soaked in tire water, then extracted with ethanol. The ethanol was dissolved in dilution water for a trout test.

Solvent extraction - Four litres were extracted by hand-shaking for one minute in a separatory funnel with 40 mL dichloromethane. The phases were allowed to separate for one hour, then the water was drained off and aerated for 24 hours to purge the dissolved solvent.

Water quality - Distilled water and dechlorinated tap water were used for dilution series tests.

Leaching - The number of tires, the volume of water and the contact time were varied.

Solvent extracts - Dichloromethane and methanol were used to extract compounds from *tire crumb*. The solvent extracts were added to water for trout tests.

Tire crumb water was made by stirring one hundred grams of tire crumb in four litres of water for twenty-four hours. A relatively low weight (tire) to volume (water) ratio was used because, compared to a whole tire, tire crumb presents a much larger surface area for toxicant partitioning into water. The tire crumb was allowed to settle for twenty-four hours, and the water was decanted for toxicity tests, chemical analyses and TIE treatments. Tire crumb or samples of tire crumb water were subjected to the following treatments for *Daphnia magna* acute lethality tests:

Filtration - A water sample was passed through a 1.2 µm glass fibre filter. This also was a pre-treatment for solid phase extraction (SPE).

SPE - A water sample was passed through a 3-mL C_{18} SPE column, and the column was eluted with 750 uL methanol.

Adjustment of pH - Tire crumb was leached with water adjusted to pH 2, 4, 10 and 11. The leachates were readjusted to pH 7.5 to 8.1 for testing. 1N solutions of hydrochloric acid and sodium hydroxide were used.

Sequential extracts - Tire crumb was leached nine times in succession with separate four litre volumes of water.

Solvent-extracted - Tire crumb was Soxhlet-extracted with dichloromethane and then leached with water.

Metal chelation - A water sample was spiked with EDTA (ethylenediaminetetraacetate), a metal-binding agent.

Samples of tire crumb water also were subjected to filtration and sequential extractions with water for *Ceriodaphnia dubia* 7-day survival and reproduction tests. The water samples were stored at 4 °C in the dark until needed to renew the toxicity test solutions during a test.

Two trout tests were conducted on a commercial automotive product used to clean and protect tire surfaces. The product is a proprietary mixture, but it is believed to contain the same alkylphenols used as protective agents in tire manufacture. Single concentrations of 1000 and 2000 parts per million (volume/volume) of the whole product were tested. The product formed a milky-white dispersion when added to water, and was found to be nonlethal at both test concentrations.

2.3 Toxicity tests

The toxicity tests were conducted from September 1993 to October 1994. Canadian national procedures were followed for acute lethality testing of rainbow trout and *Daphnia magna* (Environment Canada, 1990a; 1990b). The procedures give specifications for animal cultures, dilution water, test conditions, observations, measurements and calculations. The chronic tests of *Ceriodaphnia dubia* also followed a standard procedure (Environment Canada, 1992). Dechlorinated municipal tap water was used for animal cultures and to prepare and dilute tire water. It was an alkaline *hard* water that was temperature-adjusted and aerated to oxygen saturation before use. The animal cultures and toxicity tests had a daily photoperiod of 16 hours of fluorescent light.

The trout were hatchery fish acclimated in the laboratory for at least two weeks at 13 to 17 °C. The daphnids were from reproducing laboratory cultures at 18 to 22 °C (*D. magna*) and at 23 to 27 °C (*C. dubia*). The trout were raised in tanks with aerated flow-through water, and the daphnids were raised in static cultures with water replacement two or three times per week. The cultures were fed daily. The fish were fed commercial trout chow. *D. magna* were fed a combination of two algae species (*Selenastrum capricornutum* and *Chlorella fusca*). *C. dubia* were fed a mixture of yeast, cereal grass and trout chow supplemented twice weekly with the two-algae diet.

A sample of tire water was diluted to different ratios for toxicity testing. The dilutions are expressed as a percentage by volume. An undiluted sample of tire water is defined as the 100% concentration. 10% tire water would be diluted with 90% dechlorinated municipal tap water. The water temperature, pH, dissolved oxygen concentration and specific conductivity of the toxicity test dilutions were measured before and after the tests. The test animals were checked daily to observe survival or reproduction rates. Table 3 summarizes the test conditions.

A toxicity test was ended after a fixed exposure time, and the cumulative percent mortality was calculated for each tire water dilution. The toxicity test data were used to estimate the median lethal concentration (LC50) and 95% confidence limits for each day of exposure. Median lethal times (LT50s) and 95% confidence limits were estimated from the cumulative percent mortality over time in each tire water dilution. Low values of LC50 or LT50 denote high toxicity. The *Probit* method was used to estimate toxicity values when partial mortalities (16 to 84%) occurred, otherwise the *Spearman-Karber* method was used. The LT50s and LC50s for separate samples were considered significantly different if the confidence limits did not overlap. If confidence limits are not shown, they could not be calculated from the data. For some samples, the toxicity value is reported as *LC50 > 100%* or *LT50 > 96 hours*. These samples caused some mortality, but it was less than 50%, so the standard estimate of toxicity could not be made.

Table 3. *Toxicity test specifications.*

Test species	**trout**	***D. magna***	***C. dubia***
Exposure time (days)	4	2	7
Life stage	fry	neonates	neonates
Weight or age	0.7-4.3 g	< 24 hours	< 24 hours
Test vessel	20-L plastic bucket	50-mL glass vial	30-mL plastic cup
Solution volume	20 L	50 mL	15 mL
Loading	10 fish	3 neonates	1 neonate
Replicates	none	4	10
Temperature (°C)	13 to 17	18 to 22	23 to 27
Aeration	yes	no	no
Food	none	none	algae and YCT[1]

[1] YCT is Yeast, Cereal grass and Trout chow in aqueous suspension.

2.4 Chemical analyses

At the same time as water samples were collected for toxicity tests and TIE treatments, samples also were collected for chemical analyses for: copper, nickel, lead, zinc, iron, cadmium, chromium, ammonia, nitrates, nitrite, pH, specific conductivity, dissolved inorganic carbon, dissolved organic carbon, chloride, sulphate, hardness, alkalinity, calcium, magnesium, sodium, potassium, fluoride and total unfiltered reactive phenols, measured by the 4-anti-aminopyrine test.

Characterization analyses were conducted to identify nontarget organic compounds using gas chromatography/(full scan) mass spectrometry (GC/(FS)MS). The samples were partitioned into base-neutral and acid fractions, and extracted with dichloromethane. The reported concentrations are approximate, and were calculated relative to the internal standard, d_{10}-phenanthrene. One tire water sample was analyzed for volatile compounds by a purge-and-trap method followed by GC/(FS)MS. One sample was scanned for extractable polar organic compounds using reverse phase liquid chromatography/(particle beam) mass spectrometry.

Ultraviolet (250-400 nm) and visible (400-800 nm) light absorbence spectroscopy was used to scan tire water and tire crumb water. Tire crumb was Soxhlet-extracted with dichloromethane, and the extract was analyzed for organic functional groups by Fourier Transform Infrared Spectroscopy (FTIR).

Tire crumb was extracted with distilled water at pH 6 following the MOEE leachate procedure (as per Schedule 4, Ontario Regulation 347), and the extract was analyzed for inorganic contaminants. The tire crumb also was scanned directly for metals by an Inductively Coupled Plasma (ICP) technique, and a portion was examined by scanning electron microscopy for crystalline structures indicative of certain compounds.

3.0 RESULTS

All the data are presented in the appendices. The key data are summarized below to support the main findings.

3.1 Flow-through tire water

Appendix M lists the LT50 results of the trout tests and Appendix N gives the LC50 test results. The figures below are a compilation of the LT50 data for the tire water samples collected over time during each of the three sets of flow-through experiments:

Reef tires (Figure 1)

At the start (days 7, 11 and 21), the tire water was toxic at 1 L/min flow, but this same flow resulted in nontoxic conditions towards the end of the period (days 65, 78 and 84). Flow rates of 1.5 and 2.0 L/min produced nontoxic samples (days 30 to 60), and the toxicity was restored by lowering the flow to 0.5 L/min. The 4d-LC50 was significantly lower for the day-11 sample (45%) than for the day-21 sample (71%), although the flow was 1.0 L/min in both cases. In a trout test, toxicity was not observed until the second day of exposure, and tended to increase over the exposure period. The 30 to 40% concentrations were the highest nonlethal levels. The only mortality at 2.0 L/min occurred for the sample at day 29, whereas samples collected at day 31 and day 36 were nonlethal at this same flow rate.

No metals, phenols or organic compounds were detected in the water sample collected at the Lake Erie tire reef, and other chemical parameters were within normal limits.

Trench tires (Figure 2)

Nontoxic tire water was generally produced at flows of 0.5 and 1.0 L/min (days 5 to 16). When the flow was lowered to 0.25 L/min (days 21 to 32), lethal conditions occurred initially but the water samples tended to be less toxic (LT50 > 96 hours) towards the end of this period. A low flow of 0.16 L/min restored the lethality, but flows around 0.36 L/min were nonlethal at the end of the flow period (days 68, 69 and 78).

The sample of water suspected of having contact with the tire trench was nonlethal to trout. The chemical data are listed in Appendices B, E and L. GC/MS analysis found only traces of a few nitrogen and sulphur compounds that would be associated with tire leachate. No metals or phenols were detected, and the other parameters were within normal limits, except the water pH (9.2 and 9.4) was relatively high.

Scrap tires (Figure 3)

The sample collected on day 15 at 0.5 L/min flow was lethal, but the same flow later produced a sample that was nonlethal (day 39) and one that was LT50 > 96 hours (day 42). Higher flow rates (1.0 and 1.5 L/min) were nonlethal.

In summary, the key findings are:

- *the decrease in toxicity with the increase in water flow rate; the flow rate of 1.5 L/min per 600 L water volume produced nonlethal conditions for all tires in all tests;*

- *the decrease in toxicity over time in flowing water;*

- *the tires from the three-year-old trench were generally less toxic than the tires from the 1.5-year-old artificial reef in Lake Erie.*

The implications of these findings for tires in the aquatic environment are discussed in section 4.

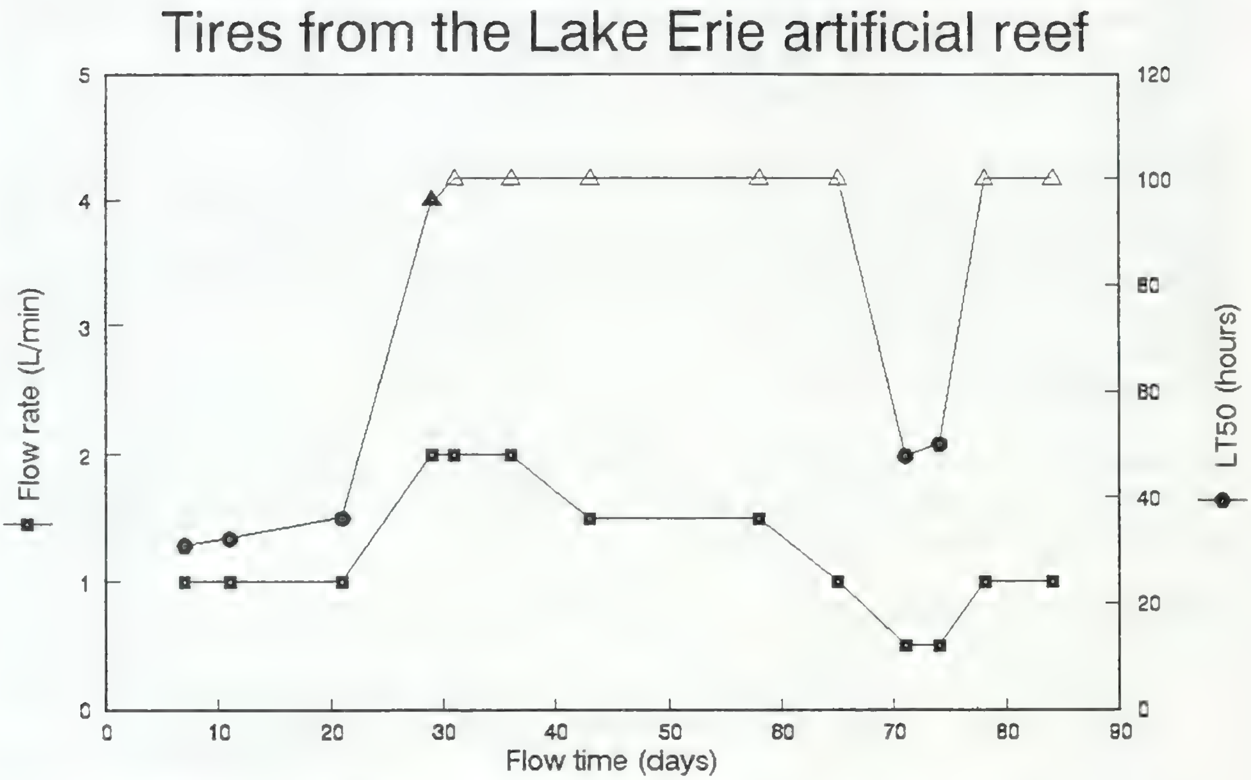

Figure 1. Flowthrough tire water toxicity vs flow rate

Legend for LT50 results:

● Lethal (greater than 50% mortality in the 96-hour test)

▲ LT50 > 96 hours (some mortality, but less than 50%)

△ Nonlethal (no fish died in the test period)

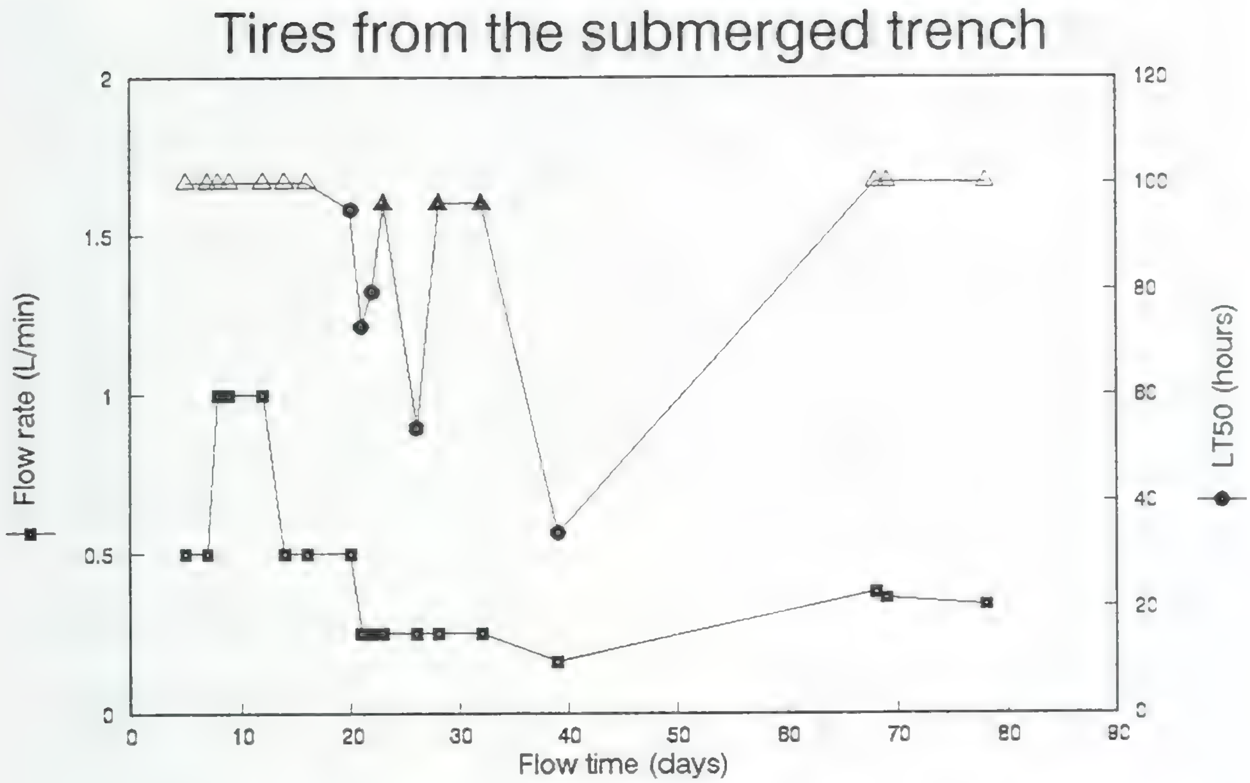

Figure 2. Flowthrough tire water toxicity vs flow rate

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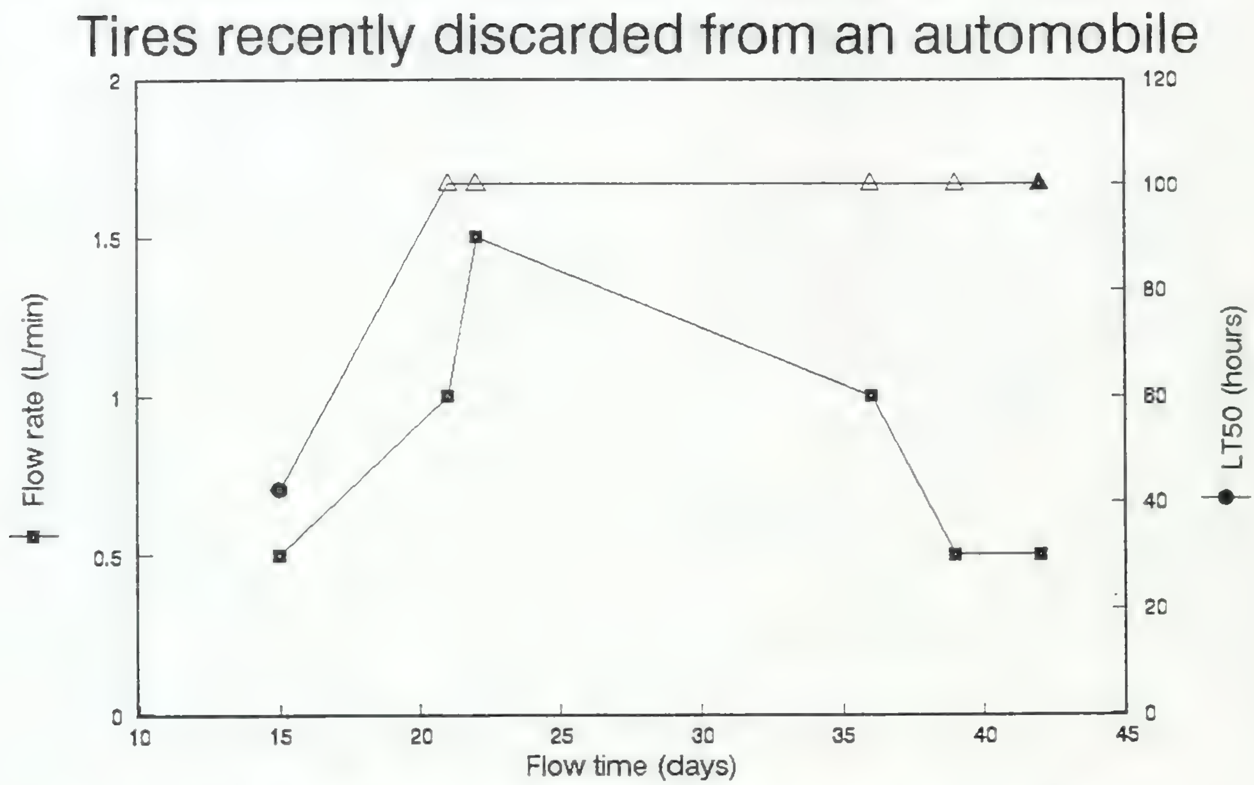

Figure 3. Flowthrough tire water toxicity vs flow rate

Legend for LT50 results:

● Lethal (greater than 50% mortality in the 96-hour test)

▲ LT50 > 96 hours (some mortality, but less than 50%)

△ Nonlethal (no fish died in the test period)

3.2 Static tire water

Appendix O gives the trout lethality data for the static tire water dilution tests. Surprisingly, tire water batch 4 was nonlethal yet the same tire had produced lethality in three previous tire water batches (see Abernethy, 1994). Just before making batch 4, the tire had been submersed in a flow-through tank containing five trout averaging 170 grams each (a separate sublethal toxicity study). During the preparation of tire water batch 4 there was a persistent layer of brown-coloured bubbles on the water surface, indicative of natural surfactants probably from organic matter and bacterial growth in the water tank.

Scrap tires leached for the first time (batch 16) were more toxic than the reef tires (batches 7 to 15). For example, batch 15 (4d-LC50 = 77%) and batch 16b (4d-LC50 = 36%) were both made by soaking tires for three days. Batch 16c had the lowest 4d-LC50 (27%) of any of the batches tested. Increasing the number of tires per volume of water and/or the submersion time increased toxicity using the reef tires (batch 8 vs 9 and batch 14 vs 15) but only slightly increased toxicity using scrap tires (batch 16b vs 16c). This suggests the scrap tires not previously submersed may have released more toxicant than the reef tires, and the amount released reached the toxicant's water solubility limit.

3.3 Toxicity Identification Evaluation

3.3.1 Static tire water

3.3.1.1 Trout lethality

A comparison of daily LC50s over the course of a trout test (Appendix O) shows that a threshold LC50 generally was reached after three days of exposure. Little difference in toxicity was found for tire water batches that differed in: makes and models of tires used or the tire surface condition (scrubbed tire, batch 7 versus unscrubbed tire, batch 8), the dilution water quality (batches 12 and 13c) and the sample age or storage time (batch 14).

A comparison of the LT50s (Appendix P) shows that baseline tests of fresh unaltered samples typically caused 100% mortality within seventeen hours of exposure. No mortality was observed in the first six to ten hours of exposure. The toxicant was relatively stable in water: Only a slight reduction in toxicity occurred from longer sample storage times. Storage temperature (15 versus 20 °C), exposure to light and the type of container (plastic versus glass) did not influence the toxicity (tests 10, 11, 13 to 16, 19 to 23, 26 and 28). Pre-aeration (tests 12 and 26) slightly reduced toxicity. Soaking the same tires for three, six or thirteen days (tests 28, 30 and 31) did not influence the LT50.

Steam distillation (test 28) partially detoxified the tire water, suggesting that a toxic component was boiled-off and thus had a boiling point less than that of water (100 °C). This could be confirmed by capturing the steam, cooling it to distilled water and conducting a toxicity test on the water. Another component of the toxicant was not boiled-off, and is therefore likely to be polar. As a class, polar compounds like many amines and phenols have higher water solubilities, lower vapour pressures and higher boiling points than nonpolar compounds of the same molecular weight.

Sulphur compounds such as benzothiazoles were prevalent in tire water, and they have been found in surface water receiving effluent from a tire manufacturing plant (Jungclaus *et al.*, 1976). However, a tire water sample was not detoxified by adding an organo-sulphur binding agent (test 25) (Russell, 1975). Tire water was partially detoxified by extraction with methylene chloride (test 26, 30 and 33), and completely detoxified by the addition of activated carbon (test 23, 24, 25 and 27). A portion of the toxicity was recovered from the contaminated carbon by solvent extraction with ethanol (test 38).

As was observed from the LC50 results, the time to mortality (LT50) was not altered significantly by dilution with distilled water compared to well-buffered, hard water (test 29 and 34). The results were confounded by the 10 to 20% control mortality in distilled water alone. Calamari *et al.* (1980) found that aliphatic amines were more toxic to trout in soft water compared to hard water. There was no evidence here implicating these substances as toxicants even though the tire water had an odour characteristic of aliphatic amines. Since distilled water differs in other water quality parameters (like pH and conductivity), the comparison was not conclusive by itself.

The Soxhlet-extract of the tire crumb was a viscous dark-brown liquid or *tire oil*. 50 mg/L of the tire oil was lethal in two trout tests (Appendix U). Subjectively, the fish responses to tire oil differed from those observed during the tire water tests. The toxicant in tire oil is probably different than that leached from tires. The methanol extract of tire crumb also was lethal to trout.

3.3.1.2 Chemical characterization

The tire and control waters were chemically similar (Appendix A) except low, nontoxic levels of zinc were found in the tire waters. Total phenols were measured at slightly higher levels in tire water batches 4, 5 and 6 than in batches 7, 10 and 13 (Appendix L). When tire water was scanned with ultra-violet and visible light (Appendix J), no significant light absorbence was found. This suggests the major components of the tire water were not aromatic, or at least not concentrated enough to be detected by this technique. However ultra-violet fluorescent spectroscopy is the preferred technique for confirming the presence of aromatic compounds.

The organic compounds detected by GC/MS are listed in Appendix D and the numbers detected are shown in Table 4. The *total* number of compounds is comprised of the numbers *identified*, *classified* and *unknown*. These three designations were based on the degree of confidence of identification. Identified compounds were known specifically (e.g. *aniline*) and with a high degree of confidence. Classified compounds could not be identified, but had specific structural features of a compound class (e.g. *an arylamine*). Unknown compounds are substances that could not be identified, and no known structural features of a compound class were evident. Control waters were found to be relatively uncontaminated. As expected, the prevalent compounds in tire waters are common rubber-processing chemicals (benzothiazoles, arylamines, alkylphenols), their impurities and breakdown products. Tire water batches 4 and 6b (scrap tires, group D) had significantly more detectable compounds than batches 7, 10 and 15 (reef tires).

Table 4. *Numbers of extractable organics in static tire water.*

Tire water batch	**Number of compounds**			
	total	**identified**	**classified**	**unknown**
4	35	17	6	12
6b	26	11	10	5
7	3	0	3	0
10	9	2	5	2
15	4	1	2	1

GC/MS for volatile organics (Appendix G) did not reveal any significant contaminants. LC/MS analysis for polar organics (Appendix H) did not find any additional chemicals except for an alkylphenol. Further LC/MS scans would be desirable because the toxicant has polar properties. However the sample preparation for this technique is too labour-intensive for routine applications.

3.3.2 Tire crumb water

3.3.2.1 *D. magna* lethality

Appendix Q lists the LC50 results for the *D. magna* tests of tire crumb water. Filtration removed a portion of the toxicity, probably associated with fine particles of tire crumb that remained suspended in the water. Nine sequential water-extractions of the same tire crumb sample (TCW-9 series) did not exhaust the toxicant, so it was probably a major constituent of the rubber. The toxicity was removed when tire crumb was first Soxhlet-extracted (TCW-extracted). When tire crumb was extracted with water at extreme pH values, then tested at the baseline pH, the toxicity was influenced significantly. The 2d-LC50s were < 5%, 24% and 78% for the pH 2, 11 and baseline extracts respectively.

When samples of tire crumb water were treated with the metal-chelating agent EDTA, the toxicity was reduced (Appendix R). This suggests a cation metal as the prime cause with an additional toxicant present. Acute lethality was reduced by the solid phase extraction (SPE) for nonpolar organic compounds. However, some samples of SPE-treated dilution water had slight toxicity, indicating a toxic artifact that confounded the results (Appendix S). The toxicant was not eluted from the SPE columns by methanol. Nelson *et al.* (1994) had similar SPE results testing water-leachate of *plugs* cut out of tires, and identified cationic metal (zinc) toxicity.

3.3.2.2 *C. dubia* survival and reproduction

Appendix T lists the results of the *C. dubia* chronic tests. 7d-LC50s and 7d-EC50s (inhibition of reproduction) were similar, ranging from 7 to 19%. The sequential extractions of the same tire crumb sample (TCW-9 series) did not exhaust the toxicant material, so it was probably a major constituent of the rubber. Water filtration had little effect on the chronic toxicity.

3.3.2.3 Chemical characterization

Tire crumb water had high concentrations of zinc and total phenols (Appendices C and L). The acidic batch, prepared at pH 2, contained 14 mg/L of zinc and 220 ug/L of total phenols, the highest levels found in any of the prepared tire waters. In the standard MOEE leachate test, only 0.28 mg/L of zinc was extracted from the tire crumb. Other inorganic metals were leached out at lower (< 100 μg/L) levels. GC/MS analyses found numerous organic compounds in tire crumb water (Table 5). Only 15 to 40% of the detectable compounds could be identified. Many benzothiazoles, arylamines and alkylphenols were present at levels about ten to one hundred times higher than in the tire water. The unknown compounds tended to have the longer chromatographic retention times that indicate higher molecular weights and lower water solubilities.

Table 5. *Numbers of extractable organic compounds in tire crumb water.*

Tire crumb water batch	Number of compounds			
	total	**identified**	**classified**	**unknown**
1	68	26	24	18
9a	111	31	31	49
pH 2[1]	92	18	26	48
pH 2	86	22	11	53
pH 11	101	27	58	16
pH 8[2]	66	10	27	29

[1] base/neutral fraction; [2] baseline pH

More organic compounds were leached out by water at extreme pH than at the baseline pH of 8. The acid fraction of the pH 2 extract contained cyclic diketones, including p-benzoquinone (CAS #106-51-4) at roughly 800 μg/L. The ketones could be rubber additives or the oxidation products of aniline- or hydroquinone-type additives. The base-neutral fraction of the pH 2 extract contained mg/L levels of aniline and several other cyclic amines. About 3 mg/L total of three resin acids was found in the pH 11 extract. As expected, the organic bases (amines) readily dissolved in the acidic water, and the organic acids (resin acids) in basic water, because the compounds would be in the dissociated (ionized) form and thus polar like water.

An examination of the white particles of tire crumb by electron microscopy suggested the presence of titanium with lesser amounts of silicon and aluminium. This was probably from the titanium oxide used for tire whitewall paint. The structures that composed the black particles of tire crumb were consistent with silicon, sulphur and zinc, and included an *unresolved envelope* of organic compounds (Appendix I). Waddell and Evans (1994) identified the same inorganic constituents using x-ray emission spectroscopy. No significant light absorbence was found when the samples of tire crumb water were scanned with ultra-violet and visible light (Appendix J).

The dichloromethane Soxhlet-extract of tire crumb was a viscous dark-brown liquid, a tire oil. FTIR spectra indicated it contained a mixture of paraffinic, naphthenic and aromatic hydrocarbons with a minor quantity of carbonyl compounds (esters and possibly acids). The spectra resembled those obtained from lubricant oils (Appendix K). The petroleum-derived hydrocarbons comprising the tire oil were not found in water extracts of tire material. With respect to the inorganic constituents of tire crumb, direct analysis by ICP found 17.6 mg/g of zinc as the primary metallic constituent.

4.0 DISCUSSION

The key results for discussion are summarized as follows:

- *The samples of flow-through tire water collected at a minimum flow rate of 1.5 L/min per 600-litre water volume were nonlethal to trout for all tires in all tests.*

In the flow-through experiments, the minimum flow that produced nonlethal conditions corresponds to a tank *flushing time* of about 15 to 20 hours for 90% replacement of the water volume with uncontaminated in-coming water (calculated following Sprague (1969)). Ontario surface waters vary in flow characteristics, but most provide enough dilution to prevent lethality around tire structures.

- *The samples of flow-through tire water collected at the end of a submersion period were less toxic than those collected at the beginning, for the same water flow rate.*

There are several explanations for the decrease in the rate of chemical release during the tire submersion periods. Over time, the tires probably were depleted of chemical substances by a continuous process of leaching. Chemical release also may have been reduced by a bacterial growth that formed on the surface of the tires during the submersion periods. The composition of the rubber at the tire surface also may have changed over time in the flowing water, possibly affecting chemical release.

- *Under static water conditions, the reef tires were less toxic than the scrap tires that were placed in water for the first time. Chemical analyses detected many contaminants released from the scrap tires that had not been previously submersed, but few contaminants from the reef tires.*

- *A tire that had been lethal to trout under static water conditions was placed in a tank of flowing water for two weeks. A subsequent batch of static tire water was nonlethal.*

Previous exposure to an aquatic environment probably had depleted the reef tires of potential toxicant and other detectable chemicals due to a continuous process of leaching. However, other processes (for example, a change in the composition of the tire rubber) may be involved in reducing the toxicity of previously submersed tires. Day *et al.* (1993) found that tires collected from a ten-year-old floating breakwall in Lake Ontario were nonlethal to trout in comparable static test conditions.

- *The tires from the 3-year-old tire trench site tended to be less toxic in flowing water than the tires from the 1.5-year-old Lake Erie artificial reef.*

This point is difficult to explain because the tires in the covered trench were in contact with ground water, but were not exposed to an aquatic environment *per se*. The difference in toxicity was slight between these two groups of tires, so the finding may not be significant.

The results of the toxicity identification evaluation suggest that the tire water toxicant was a nonvolatile mixture of polar and nonpolar organic compounds. Latawiec (1994) also concluded that the toxicant is a mixture of polar and nonpolar compounds. The higher ratio of polar to nonpolar toxicants leached from scrap tires (1:1) compared to new tires (1:4) would explain why the leachate from scrap tires is more toxic to trout than leachate from new tires (Day *et al.*, 1993). As tires age, polar oxygen-containing breakdown products are formed from the parent rubber compounds.

The specific chemical cause of the tire water toxicity remains unknown. Researchers at the National Water Research Institute published a series of papers investigating tire water leachate (Anthony, 1993; Anthony and Barclay 1993; Anthony and Latawiec 1993; and Latawiec, 1994). The leachate was concentrated into solvent extracts for chemical analyses coupled with toxicity tests using the luminescence of a marine bacterium. The studies found: the solvent extracts were an intense yellow colour; the coloured compounds were high molecular weight, polar and easily oxidized suggesting aldehydes, quinones and nitrogen heterocyclic groups; many of the organic chemicals can be classified as conjugated heterocyclics; an aliphatic nitro-species attached to a 5-member ether heterocycle was a main component; and bacterial luminescence was affected primarily by the 75 to 80% methanol SPE extracts.

In the present study, a different mixture of chemicals was detected by GC/MS in every tire water sample analyzed. One reason would be the different compounding recipes used to manufacture various tires. Also, the compounds in a tire degrade and transform as the tire ages by oxidation and other processes (Hofmann, 1989). Nevertheless, a few chemicals and chemical classes were predominant in tire water. These are the ones widely used in rubber compounding recipes.

Likewise, the specific toxicant mixture might vary for different tires and differ for the same tire over time, but it is believed that most of the toxicity can be attributed to *principal components* from a few chemical classes widely used in the manufacture of all tires. This is consistent with the finding (Day *et al.*, 1993) that toxicant leaching in water has been found for all makes and models of new and used tires that have been tested.

The search for the identity of the toxicant is complicated because about twenty thousand chemicals are known in the rubber industry. Hofmann (1989) provides an extensive technical review of the industry and the chemical compounds involved. The compounding ingredients used to make synthetic rubber occur in proprietary formulations that may lose their identity after they are mixed and reacted. The first suspects as toxicants would be the chemicals used in the largest amounts, the accelerators, fillers, plasticizers and protective agents.

The present findings implicate (aromatic) amine compounds as the principal toxicants. The tire water colour, odour, chemical composition and the TIE results were all consistent with the physical-chemical and toxicological properties of amines. The tire crumb water (pH 2 extract) contained aniline at a level almost one hundred times higher than a typical acute LC50 for *D. magna* (0.5 mg/L) reported in the literature. The extract also contained about 26 mg/L total of six other amines. As a chemical class, aromatic amines have significant environmental hazards (MacLaren, 1980; Fishbein, 1991). Some are easily oxidized to para-benzoquinone, also found in the pH 2 extract. Para-quinones and hydroquinone, a prevalent reduced form, are very toxic to fish. Verschueren (1983) reported 48-hour *approximate fatal concentrations* to goldfish (*Carassius auratus*) of 0.28 mg/L for hydroquinone and 6 mg/L for p-phenylenediamine. Yoshioka *et al.* (1986) reported a 48-hour LC50 for red killifish (*Orizias latipes*) of 20 mg/L for p-phenylenediamine and 2 mg/L for diphenylamine.

Aromatic amines may comprise up to three percent by weight of an automobile tire. Derivatives of p-phenylenediamine are the most common additives used to protect rubber from aging and weathering. The protective action (and toxicity) of amines can be altered by changing their molecular size using different substituents (attached groups). For example, the largest molecules hardly migrate in rubber so they are good anti-oxidants, but their immobility makes them poor anti-ozonants. Smaller molecules are effective anti-ozonants because they can continually rise to the tire surface where the protective action is required. The smallest-sized phenylenediamines are not used in tires because they migrate too fast and are rapidly lost by direct volatilization and by a water-leaching effect. They are also known to cause dermatological effects (Hofmann, 1989). Chemical migration out of a tire implies that the concentrations at the tire surface eventually would be depleted and the toxicant release rate would decrease over time, as was inferred from the trout test results of tire water.

The tire crumb material leached zinc at levels above known toxicity values, and thus zinc probably was the major toxicant to *D. magna* and *C. dubia* in the tire crumb water tests. In the literature, 0.8 mg/L zinc is a typical *D. magna* acute LC50, and 0.1 mg/L is a typical chronic toxicity value for *C. dubia*. Nelson *et al.* (1994) identified zinc as the toxicant in plugs cut out of whole tires. However whole tires did not leach zinc at toxic levels in the present study. Zinc is a key ingredient in rubber-compounding recipes. The high lethality to *D. magna* and low zinc level of the pH 11 extract and the partial removal of toxicity by the EDTA treatment suggest the presence of an additional, organic toxicant.

The resin acid (diterpenoid carboxylic acids) levels found in the pH 11 extract approximate acute toxicity values for daphnid species. Large amounts of resin acids (2 to 7% of the rubber) may be used as plasticizers and emulsifiers in rubber compounding. They are only partially removed during washing, so some of the residues occur in the finished products (Hofmann, 1989). Salmonid species are sensitive to the acute effects of both resin acids and tire water. The resin acids are nonvolatile like the tire water toxicant, but none were detected in a previous study (Abernethy, 1994). Many high molecular weight aromatic and aliphatic carboxylic (fatty) acids are known rubber plasticizers that merit further consideration as potential tire water toxicants.

Alkylphenol compounds are used in large amounts in tire manufacture, many were detected in tire water and as a group they are nonvolatile like the toxicant. However, a commercial product containing alkylphenols was nonlethal to trout at high concentrations of the product, so there is less reason to suspect them as toxicants based on these results. Many phenolic compounds form coloured-complexes with ferric chloride. This suggests a simple colorimetric measure of phenols and a method to remove them from solution.

If a further investigation of the toxic constituents is conducted, it should be limited to identify a few specific, representative compounds (for example, certain aromatic amines). Then samples of ambient water around a tire structure could be collected, and the chemical levels compared to surface water quality standards to help make decisions about placing tires in natural water bodies. For this, an analytical method is required to separate and identify mixtures of polar and water-miscible compounds. Appropriate specialized procedures using liquid chromatography are being developed and should be more widely available within a few years. Some of the toxicant was extractable in methylene chloride, and the tire crumb water provided an enriched source of leachate especially at extreme pH. Toxicity tests conducted on pH-adjusted, solvent-extracted tire crumb water might be able to isolate the toxicant in a concentrated form for easier identification. Likewise, Soxhlet-extractions with other solvents, such as hot water, might be able to generate large amounts of the toxicant.

Large amounts of carbon black, saturated with polynuclear aromatic hydrocarbons and sulphur heterocyclic compounds, are used as filler in rubber tires. Carbon black has a wide environmental distribution in automobile tire dust (Lee and Hites, 1976), but only traces of a few sulphur heterocyclics were detected in tire water. It is probable that carbon black compounds are generally immobile in tires placed in water. The carbon black is not the same as the activated carbon that detoxified the static tire water in the trout tests. Activated carbon has a very high surface area to volume ratio, many binding sites and thus a large capacity to sorb chemicals, removing them from water.

Lerner *et al.* (1993) investigated potential chemical contamination from shredded tires used as a substitute for gravel aggregate in domestic septic drainage fields. The laboratory study noted that microbial activity was eventually induced after a month of soaking shredded tires in water (indicating biodegradation). Laboratory leachates were prepared in waters adjusted to various pH and ionic strengths, and leachates were collected from a pilot septic drainage field. The typical rubber compounds, zinc and benzothiazoles, were leached from tire shreds regardless of the water quality of the laboratory leachates. Fewer contaminants were found in the septic samples. The authors recommended: (shredded) tires should not be used in ways that offer a clear path to groundwater; controlled and monitored demonstration projects would be necessary before permitting environmental usage; and storage of tires for six to twelve months in open lagoons may render them more acceptable for environmental uses.

The Minnesota Pollution Control Agency investigated the potential environmental impacts of scrap tire pieces used as sub-grade material for roadway support over wetlands. Studies were conducted to identify metals and hydrocarbons leached from tires into water and soil under laboratory and field conditions (TCTC, 1990). A limited number of soil samples and a terrestrial vegetation survey did not show any differences between a tire site and a control site. Elevated levels of metals were found in a groundwater sample collected beneath a road section with a tire sub-grade compared to a sample beneath another section without a tire sub-grade. The authors recommended that tires should be placed only in the unsaturated zone of the road sub-grade, and that such roads should be designed to limit infiltration of water through the sub-grade.

5.0 CONCLUSIONS

Overall the evidence suggests that tires in Ontario waters are unlikely to cause acute lethality to trout and other aquatic life primarily because relatively low rates of water flow can provide sufficient dilution to prevent the effect.

The flow-through tests showed that the scrap tires were nonlethal to trout at a minimum water flow rate of 1.5 L/min per 600 L water volume, a flow less than that provided by most Ontario surface waters. Other natural processes (for example, biodegradation, photolysis and particle-binding) also may reduce the potential for toxicity in waters around a tire structure. Bench-top treatments of tire water using coagulants and flocculants, ultra-violet light and biological treatment processes would provide more information on these processes and their importance for toxicity reduction.

The decrease in the rate of chemical release during each tire submersion period in the flow-through tests was probably due to a continuous process of leaching that depleted the tires of chemical substances. The chemical release rate also may have been reduced by a bacterial growth that formed on the surface of the submersed tires. The composition of the rubber at the tire surface also may have changed over time, possibly affecting chemical release.

The static tests showed that tires collected from the artificial reef in Lake Erie were less toxic than scrap tires that had not been previously exposed to an aquatic environment. Chemical analyses of the static tire waters detected many more contaminants released by the scrap tires than by the reef tires. Previous exposure to an aquatic environment probably had depleted the reef tires of potential toxicant and other detectable chemicals due to a continuous process of leaching. Before tires are placed in surface waters, leaching (for example, storage in lagoons for six to twelve months) and treatment of the leachate would ensure that potential toxicants and other contaminants are not being directly released to the aquatic environment.

The toxicant in static tire water was characterized as a nonvolatile mixture of polar and nonpolar organic compounds, but the specific constituents were not identified. Aromatic amines were suspected as the principal component of the toxicant. The complete identification of all the toxic constituents released by tires would be practically impossible due to the large numbers of compounds used in tire manufacture and the chemical reactions that occur as tires age.

If a further investigation of the toxic constituents is conducted, it should be limited to identify a few specific, representative compounds (for example, certain aromatic amines). Then samples of ambient water around a tire structure could be collected, and the chemical levels compared to surface water quality standards to help make decisions about placing tires in natural water bodies. Although trout lethality tests were appropriate for the present investigation, the use of more sensitive chronic sublethal tests would be the next logical step for further studies at lower, more realistic levels of exposure.

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APPENDIX A

ANALYSES OF TIRE WATER (TW) AND CONTROL WATER (CW)

Water concentrations are mg/L, except metals are ug/L. Nitrogen compounds as N. Carbon = dissolved carbon as C. UNF = unfiltered, R = reactive, T = total, nd = not detected. The detection limits were: ammonium 0.05; nitrite 0.015; copper 1.9; nickel 1; lead 8; zinc 7; iron 14; cadmium 0.2; chromium 1.

	Batch #4		Batch #5		Batch #6		Batch #7	
Chemical parameter	**CW**	**TW**	**CW**	**TW**	**CW**	**TW**	**CW**	**TW**
conductivity, µmho/cm	332	331	333	333	337	339	344	362
hardness, T, as $CaCO_3$	131	135	130	131	132	132	141	144
calcium, UNF,R	38	39	37	38	39	39	40	41
magnesium, UNF,R	9	9	9	9	9	9	10	10
sodium, UNF,R	13	13	13	13	12	11	13	13
potassium, UNF,R	2	2	2	2	2	2	2	2
alkalinity, T, as $CaCO_3$	94	94	91	92	95	96	101	102
pH	8.4	8.3	8.2	8.2	8.2	8.1	8.4	8.4
fluoride, UNF,R	1	1	1	1	1	1	1	1
chloride, UNF,R	28	28	27	27	26	26	26	27
sulphate, UNF,R	33	33	32	32	32	32	32	32
ammonium, frac. R,T	nd	nd	nd	nd	nd	nd	nd	nd
nitrite, frac. R	0.03	nd	nd	nd	nd	nd	nd	nd
carbon, organic	1	1	2	3	2	2	2	2
carbon, inorganic	21	21	20	20	21	22	24	23
copper, UNF,T					3	2	4	4
nickel, UNF,T					nd	nd	nd	nd
lead, UNF,T					nd	nd	nd	nd
zinc, UNF,T					nd	30	nd	nd
iron, UNF,T					nd	nd	68	nd
cadmium, UNF,T					nd	nd	nd	nd
chromium, UNF,T					nd	nd	17	nd

APPENDIX A

ANALYSES OF TIRE WATER (TW) AND CONTROL WATER (CW)

Chemical parameter	Batch #10		Batch #13		Batch #15	
	CW	TW	CW	TW	CW	TW
conductivity, μmho/cm	366	362	360	356	324	337
hardness, T, as $CaCO_3$	147	145	142	142	131	138
calcium, UNF,R	43	43	41	42	39	41
magnesium, UNF,R	9	9	9	9	8	9
sodium, UNF,R	15	14	15	15	14	15
potassium, UNF,R	2	2	2	2	2	2
alkalinity, T, as $CaCO_3$	99	99	99	98	91	97
pH	8.3	8.2	8.2	8.2	8.0	8.0
fluoride, UNF,R	1	1	1	1	1	1
chloride, UNF,R	29	28	30	29	26	27
sulphate, UNF,R	37	36	32	33	30	29
ammonium, frac. R,T	nd	nd	nd	nd	nd	nd
nitrite, frac. R	nd	nd	0.03	nd	nd	nd
carbon, organic	2	2	2	2	1	2
carbon, inorganic	22	22	22	22	21	22
copper, UNF,T	nd	nd	nd	2	nd	nd
nickel, UNF,T	nd	nd	nd	nd	nd	nd
lead, UNF,T	nd	nd	nd	nd	nd	nd
zinc, UNF,T	nd	27	nd	38	nd	26
iron, UNF,T	nd	nd	37	140	39	140
cadmium, UNF,T	nd	nd	nd	nd	nd	nd
chromium, UNF,T	nd	nd	nd	nd	nd	nd

APPENDIX B

ANALYSES OF SURFACE WATERS

Chemical parameter	Lake Erie Turkey Point	Tire trench Control Pond	North End Trench tire site
conductivity, µmho/cm		252	192
hardness, T, as $CaCO_3$		145	109
calcium, UNF,R		18	14
magnesium, UNF,R		25	18
sodium, UNF,R		nd	nd
potassium, UNF,R		nd	nd
alkalinity, T, as $CaCO_3$		132	105
pH		9.4	9.2
fluoride, UNF,R		nd	nd
chloride, UNF,R	15	3	nd
sulphate, UNF,R	24	11	4
ammonium, frac. R,T	nd	nd	nd
nitrite, frac. R	nd	nd	nd
carbon, organic	2	8	7
carbon, inorganic	21	26	22
copper, UNF,T	nd	nd	nd
nickel, UNF,T	nd	nd	nd
lead, UNF,T	nd	nd	nd
zinc, UNF,T	nd	nd	nd
iron, UNF,T	32	100	60
cadmium, UNF,T	nd	nd	nd
chromium, UNF,T	nd	nd	nd

APPENDIX C

ANALYSES OF TIRE CRUMB WATER (TCW) AND CONTROL WATER (CW)

	Batch #9a		pH 8		pH 11	pH 2
Chemical parameter	CW	TCW	CW	TCW	TCW	TCW
conductivity, μmho/cm	325	346				
hardness, T, as $CaCO_3$	134	134				
calcium, UNF,R	40	40				
magnesium, UNF,R	9	9				
sodium, UNF,R	13	17				
potassium, UNF,R	1	2				
alkalinity, T, as $CaCO_3$	92	97				
pH	8.1	7.9				
fluoride, UNF,R	1	1				
chloride, UNF,R	26	28				
sulphate, UNF,R	27	32				
ammonium, frac. R,T	nd	0.5				
nitrite, frac. R	nd	nd				
carbon, organic	1	10				
carbon, inorganic	21	22				
copper, UNF,T	nd	6	4	26	12	1000
nickel, UNF,T	nd	nd	nd	nd	nd	nd
lead, UNF,T	nd	nd	nd	nd	nd	120
zinc, UNF,T	nd	1100	nd	1100	350	14000
iron, UNF,T	nd	0.2	91	570	230	12000
cadmium, UNF,T	nd	nd	nd	nd	nd	nd
chromium, UNF,T	nd	nd	nd	nd	nd	nd

APPENDIX D

GC/MS ANALYSES FOR EXTRACTABLE ORGANIC COMPOUNDS: TIRE WATER

Notes: samples were partitioned into base-neutral and acid fractions and extracted with dichloromethane; A- = approximate concentration relative to the internal standard d_{10}-phenanthrene; (A-) = approximate concentration in control sample; R.T. = chromatographic retention time. Some of the chemicals and chemical classes found are laboratory artifacts unrelated to tire leachate. Trace contaminants known from routine laboratory operations (surfactants and plasticizers) and from the outdoor air supply were found in all water samples. Tire rubber does contain some soaps and plasticizers, but the following compound classes are believed to be extraneous: the carboxylic acids with short retention times; linear alcohol ethoxylates; and hydrocarbons including the alkanes.

TIRE WATER BATCH 4
(one tire submersed for 12 days in 300 L static aerated water)

Entry	R.T.	Conc. / Identity	CAS No.
1	11.67	A-0.3 Aniline	000062-53-3
2	13.48	A-1 A methyl benzenamine	-----------
3	13.95	A-0.4 2-Pentanamine, N-ethyl-4-methyl-	42966-64-3
4	14.36	A-0.5 A nitrogen compound	-----------
5	14.80	A-0.2 1,3-Pentanediol, 2,2,4-trimethyl-	000144-19-4
6	15.13	A-0.2 Unidentified	-----------
7	15.56	A-0.2 Morpholine, 4-acetyl-	001696-20-4
8	16.26	A-2 (A-0.3) Benzothiazole (8CI9CI)	000095-16-9
9	16.63	A-0.4 (A-0.4) Formamide, N-cyclohexyl-	000766-93-8
10	19.80	A-7 1,5-Ditertbutyl-3,3-dimethylbicyclo[3.1.0]Hexan-2-one	-----------
11	20.39	A-0.4 Unidentified	-----------
12	20.90	A-0.3 Morpholine, 4-acetyl-	001696-20-4
13	20.99	A-2 A dibutylthiophene	-----------
14	21.42	A-0.3 Diethyl phthalate	000084-66-2
15	21.82	A-0.3 Benzothiazole, 2-(methylthio)-	000615-22-5
16	21.90	A-0.3 Benzenamine, N-phenyl-	000122-39-4

TIRE WATER BATCH 4 - continued.

Entry	R.T.	Conc. / Identity	CAS No.
17	22.43	A-1 Benzenamine, N-methyl-N-phenyl-	000552-82-9
18	22.51	A-2 2(3H)-Benzothiazolone	000934-34-9
19	22.62	A-0.6 A chlorine compound	-----------
20	23.06	A-0.6 A phenyl-propyl phenol	-----------
21	23.46	A-0.6 (A-0.4) A chlorophosphate	-----------
22	23.56	A-0.3 Unidentified	-----------
23	23.65	A-0.8 A C10-alkyl phenol	004130-42-1
24	23.86	A-0.3 Benzyl alcohol, α-isobutyl-2,4,5-trimethyl-	010425-87-3
25	23.93	A-0.3 Unidentified	-----------
26	24.73	A-0.7 Phenol, 4-(1-methyl-1-phenylethyl)-	000599-64-4
27	25.18	A-0.3 Unidentified	-----------
28	25.47	A-0.4 Unidentified	-----------
29	25.54	A-0.4 Unidentified	-----------
30	25.82	A-0.3 Unidentified	-----------
31	26.05	A-0.3 Unidentified	-----------
32	26.66	A-2 Morpholine, 4-(2-benzothiazolylthio)-	000102-77-2
33	28.33	A-0.8 Unidentified	-----------
34	29.85	A-0.5 Unidentified	-----------
35	31.54	A-0.3 Unidentified	-----------

TIRE WATER BATCH 6b

(three tires submersed for 13 days in 280 L static aerated water)

Entry	R.T.	Conc./ID	CAS No.
1	6.083	A-2 2-Pentanone, 4-methyl-	000108-10-1
2	9.619	A-3 (A-3) Ethanol, 2-butoxy-	000111-76-2
3	11.51	A-0.7 (A-1) An alcohol/ether	-----------
4	11.57	A-1 (A-2) An alcohol/ether	-----------
5	12.05	A-0.8 Unidentified	-----------
6	12.94	A-6 A methyl benzenamine	-----------
7	13.06	A-0.6 1,2-Cyclohexanedione	000765-87-7
8	13.40	A-0.6 An amine	-----------
9	13.81	A-0.9 Unidentified	-----------
10	15.68	A-30 Benzothiazole	000095-16-9
11	17.96	A-1 Phenol, 4-chloro-3,5-dimethyl-	000088-04-0
12	19.20	A-30 A phenol	-----------
13	19.78	A-0.9 Unidentified	-----------
14	20.37	A-8 A C8-alkyl thiophene	-----------
15	20.81	A-1 Diethyl phthalate	000084-66-2
16	21.18	A-0.6 Benzothiazole, 2-(methylthio)-	000615-22-5
17	21.28	A-2 Benzenamine, N-phenyl-	000122-39-4
18	21.79	A-2 Benzenamine, 2-methyl-N-phenyl-	001205-39-6
19	21.85	A-3 2(3H)-Benzothiazolone	000934-34-9
20	22.40	A-0.7 A phenol	-----------
21	23.03	A-2 A phenol	-----------
22	24.10	A-0.6 Unidentified	-----------

TIRE WATER BATCH 6b - continued.

Entry	R.T.	Conc./ID	CAS No.
23	25.98	A-4 Morpholine, 4-(2-benzothiazolylthio)-	000102-77-2
24	27.67	A-1 Unidentified	-----------
25	29.91	A-1 A silicon compound	-----------
26	30.94	A-1 A silicon compound	-----------

TIRE WATER BATCH 7
(one tire submersed for 13 days in 300 L static aerated water)

Entry	R.T.	Conc.ID	CAS No.
1	17.85	A-0.4 A carboxylic acid ester	-----------
2	19.20	A-0.6 A phenol	-----------
3	21.03	A-0.4 A tetramethylbutyl phenol	-----------

TIRE WATER BATCH 10
(three tires submersed for 15 days in 300 L static aerated water)

Entry	R.T.	Conc.	CAS No.
1	15.58	A-0.8 (A-0.7) Benzothiazole	000095-16-9
2	16.73	A-0.5 (A-0.3) A phenol	-----------
3	17.18	A-0.1 (A-0.1) Unidentified	-----------
4	19.09	A-1 A phenol	-----------
5	19.81	A-0.5 A nitrogen compound	-----------
6	20.76	A-0.5 A nitrogen compound	-----------
7	20.93	A-0.3 A tetramethylbutyl-phenol	-----------
8	21.09	A-0.2 Benzothiazole, 2-(methylthio)-	000615-22-5
9	24.74	A-0.6 Unidentified	-----------

TIRE WATER BATCH 15

(five tires submersed for 3 days in 600 L static aerated water)

Entry	R.T.	Conc. Identity	CAS No.
1	15.19	A-0.4 Benzothiazole	000095-16-9
2	17.10	A-0.6 An ester	-----------
3	17.36	A-0.5 An ester	-----------
4	31.77	A-0.3 Unidentified	-----------

APPENDIX E

GC/MS ANALYSES FOR EXTRACTABLE ORGANIC COMPOUNDS: SURFACE WATERS

LAKE ERIE, TURKEY POINT TIRE REEF

No extractable organic compounds were detected in the water sample collected from the tire reef.

NORTH END TIRE TRENCH

Entry	R.T.	Conc. / Identity	CAS No.
1	8.578	A-0.6 A nitrogen compound	-----------
2	12.71	A-0.4 A nitrogen compound	-----------
3	13.10	A-1 A nitrogen compound	-----------
4	14.30	A-0.8 Unidentified	-----------
5	14.40	A-0.4 A nitrogen compound	-----------
6	14.92	A-3 Benzothiazole	000095-16-9
7	15.43	A-2 Unidentified	-----------
8	15.52	A-0.9 Unidentified	-----------
9	16.29	A-0.4 A nitrogen compound	-----------
10	19.16	A-0.5 Unidentified	-----------
11	19.60	A-1 A C8-alkyl-thiophene	-----------
12	19.69	A-0.5 Unidentified	-----------
13	19.93	A-0.5 Benzamide, N,N-diethyl-3-methyl-	000134-62-3
14	20.40	A-0.3 Unidentified	-----------
15	20.75	A-0.6 An unsaturated hydrocarbon	-----------
16	20.95	A-0.8 Unidentified	-----------
17	21.13	A-0.9 Unidentified	-----------
18	21.28	A-2 An alkane	-----------

NORTH END TIRE TRENCH - continued.

Entry	R.T.	Conc. Identity	CAS No.
19	25.16	A-2 1,2-Benzisothiazole, 3-(4-morpholinyl)-	Provisional ID[1]
20	26.92	A-4 A silicon compound	-----------
21	29.14	A-8 A silicon compound	-----------
22	30.17	A-1 A silicon compound	-----------
23	32.10	A-0.3 Unidentified	-----------

[1] A check of other mass spectral databases, and further mass spectrum determinations showed that 4-(2-benzothiazolythio)-morpholine was mis-identified in the reference database, and thus in two previous analyses (tire water batch 4 and 6) and in the analyses reported previously in Abernethy (1994). This compound, eluting at about 25.16 minutes, is now identified provisionally as 3-(4-morpholinyl)-1,2-benzisothiazole.

APPENDIX F

GC/MS ANALYSES FOR EXTRACTABLE ORGANIC COMPOUNDS: TIRE CRUMB WATER

TIRE CRUMB WATER BATCH 1
(4-L aqueous extract of 100 grams of 30 to 40 mesh size tire granules)

Entry	R.T.	Conc. / Identity	CAS No.
1	5.997	A-20 2-Pentanone, 4-methyl-	000108-10-1
2	6.040	A-30 Unidentified	-----------
3	6.592	A-1 Unidentified	-----------
4	8.503	A-20 2-Hexanone, 5-methyl-	000110-12-3
5	8.708	A-40 Unidentified	-----------
6	9.293	A-10 Cyclohexanone	000108-94-1
7	9.542	A-50 Ethanol, 2-butoxy-	000111-76-2
8	10.38	A-9 2-Hexanamine, 5-methyl-	028292-43-5
9	10.66	A-30 Unidentified	-----------
10	11.02	A-30 Aniline	000062-53-3
11	11.96	A-10 A hydrocarbon	-----------
12	12.73	A-5 A hydrocarbon	-----------
13	12.85	A-10 A methyl-benzeneamine	-----------
14	13.08	A-6 Unidentified	-----------
15	13.34	A-8 A nitrogen compound	-----------
16	13.72	A-30 Unidentified	-----------
17	14.03	A-200 Hexanoic acid, 2-ethyl-	000149-57-5
18	14.14	A-40 Unidentified	-----------
19	14.76	A-20 A methyl ester of an ethyl hexanoic acid	-----------
20	14.86	A-50 A tetramethyl thiopyran + an acid	-----------

TIRE CRUMB WATER BATCH 1 - continued.

Entry	R.T.	Conc. / Identity	CAS No.
21	14.99	A-40 A methyl ester	-----------
22	15.17	A-9 A methyl ester	-----------
23	15.30	A-10 Benzoic acid	000065-85-0
24	15.65	A-400 Benzothiazole	000095-16-9
25	15.96	A-300 A carboxylic acid	-----------
26	16.07	A-200 Formamide, N-cyclohexyl-	000766-93-8
27	16.15	A-60 A methyl ester	-----------
28	16.22	A-100 A methyl ester	-----------
29	16.58	A-600 A methyl ester	-----------
30	16.73	A-200 An alkane	-----------
31	16.80	A-10 A methyl ester	-----------
32	16.84	A-10 Unidentified	-----------
33	16.96	A-20 Formamide, N-phenyl-	000103-70-8
34	17.23	A-30 A hydrocarbon	-----------
35	17.38	A-20 A dimethoxyphenol	-----------
36	17.51	A-10 An ester	-----------
37	17.66	A-5 Unidentified	-----------
38	17.78	A-10 An ester	-----------
39	18.37	A-5 A hydrocarbon	-----------
40	18.41	A-10 A hydrocarbon	-----------
41	18.66	A-600 Cyclohexanamine, N-cyclohexyl-	000101-83-7
42	18.88	A-80 1H-Indole, 2,3-dihydro-1,3,3-trimethyl-2-methylene	000118-12-7

TIRE CRUMB WATER BATCH 1 - continued.

Entry	R.T.	Conc.	CAS No.	Identity
43	19.14	A-200	000085-41-6	1H-Isoindole-1,3(2H)-dione
44	19.27	A-10	-----------	A hydrocarbon
45	19.39	A-10	-----------	Unidentified
46	19.77	A-20	007560-83-0	Cyclohexanamine, N-cyclohexyl-N-methyl-
47	19.86	A-20	007507-89-3	Ethanone, 1-(2,6-dihydroxy-4-methoxyphenyl)-
48	20.24	A-5	-----------	Unidentified
49	20.31	A-40	054845-33-9	Thiophene, 2,5-bis(2-methylpropyl)-
50	20.75	A-10	000084-66-2	Diethyl phthalate
51	20.83	A-5	-----------	A hydrocarbon
52	20.98	A-8	-----------	A C8-alkylphenol
53	21.02	A-4	-----------	Unidentified
54	21.11	A-8	000615-22-5	Benzothiazole, 2-(methylthio)-
55	21.21	A-10	000122-39-4	Benzenamine, N-phenyl
56	21.48	A-9	-----------	Unidentified
57	21.67	A-8	-----------	Unidentified
58	21.71	A-2	000552-82-9	Benzenamine, N-phenyl-N-methyl
59	21.95	A-200	000934-34-9	2(3H)-Benzothiazolone
60	22.45	A-3	000483-78-3	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-
61	22.53	A-4	-----------	A linear alcohol ethoxylate
62	22.86	A-20	-----------	Unidentified
63	23.23	A-3	-----------	Unidentified
64	24.63	A-3	-----------	Unidentified

TIRE CRUMB WATER BATCH 1 - continued.

Entry	R.T.	Conc. / Identity	CAS No.
65	24.75	A-2 1H-Carbazole, 2,3,4,4a-tetrahydro-4a-methyl-	018781-72-1
66	25.89	A-20 Morpholine, 4-(2-benzothiazolylthio)-	000102-77-2
67	26.12	A-2 Urea, N,N'-dicyclohexyl-	002387-23-7
68	29.84	A-10 Unidentified	-----------

TIRE CRUMB WATER BATCH 9A

(4-L aqueous extract of 100 grams of 30 to 40 mesh size tire granules)

Entry	R.T.	Conc. / Identity	CAS No.
1	5.43	A-70 2-Pentanone, 4-methyl-	000108-10-1
2	5.57	A-10 Unidentified	-----------
3	5.82	A-3 2-Hexanol	000626-93-7
4	6.06	A-4 2-Propanamine, N-methyl-N-(1-methylethyl)-	010342-97-9
5	7.91	A-10 2-Hexanone, 5-methyl-	000110-12-3
6	8.20	A-7 Unidentified	-----------
7	8.56	A-10 Formamide, N(1,1-dimethylethyl)-	002425-74-3
8	8.66	A-20 Cyclopentanone, 3-methyl-	001757-42-2
9	8.94	A-10 Ethanol, 2-butoxy-	000111-76-2
10	10.16	A-30 Cyclohexylamine	000108-91-8
11	10.43	A-40 Aniline	000062-53-3
12	10.49	A-10 Phenol	000108-95-2
13	11.39	A-10 Hydrocarbon	-----------
14	11.62	A-2 Hydrocarbon	-----------
15	12.16	A-3 Ethanone, 1-phenyl-	000098-68-2

TIRE CRUMB WATER BATCH 9A - continued.

Entry	R.T.	Conc.	CAS No.	Identity
16	12.27	A-10	-----------	A methyl aniline
17	12.50	A-7	000617-94-7	Benzenemethanol, α,α-dimethyl
18	12.78	A-10	-----------	A nitrogen compound
19	13.03	A-5	-----------	A carboxylic acid
20	13.15	A-30	-----------	Unidentified
21	13.48	A-200	-----------	A carboxylic acid
22	13.59	A-40	-----------	Unidentified
23	13.69	A-8	003302-10-1	Hexanoic acid, 3,5,5-trimethyl-
24	13.86	A-3	-----------	A methyl ester of an ethyl hexanoic acid
25	14.20	A-20	-----------	A methyl ester of an ethyl hexanoic acid
26	14.30	A-40	-----------	Unidentified
27	14.45	A-10	-----------	A methyl ester of a carboxylic acid
28	15.05	A-300	000095-16-9	Benzothiazole
29	15.32	A-100	-----------	An ester
30	15.45	A-90	-----------	An ester
31	15.51	A-40	000766-93-8	N-Cyclohexyl formamide
32	15.60	A-90	-----------	An ester
33	15.92	A-200	-----------	An ester
34	15.98	A-40	-----------	An ester
35	16.04	A-80	-----------	An ester
36	16.26	A-5	-----------	Hydrocarbon
37	16.38	A-10	-----------	A methyl pyridine
38	16.47	A-5	-----------	Unidentified

TIRE CRUMB WATER BATCH 9A - continued.

Entry	R.T.	Conc. / Identity	CAS No.
39	16.51	A-5 An ester	-----------
40	16.57	A-8 An ester	-----------
41	16.78	A-9 Unidentified	-----------
42	16.95	A-6 Pyridine, 3-(1-methyl-2-pyrrolydinyl)-,(S)-	000054-11-5
43	17.16	A-2 Unidentified	-----------
44	17.20	A-5 A methyl ester of a hydroxy acid	-----------
45	17.88	A-2 Unidentified	-----------
46	18.02	A-200 Bicyclo[2.2.1]heptan-2-amine, N,N,2,3,3-pentamethyl	003570-07-8
47	18.28	A-20 Quinoline, 1,2-dihydro-2,2,5-trimethyl-	000147-47-7
48	18.45	A-40 A linear alcohol ethoxylate	-----------
49	18.55	A-50 Phenol, (1,1-dimethylethyl)-4-methoxy-	025013-16-5
50	18.81	A-3 A hydroxymethoxyphenyl ethanone	-----------
51	19.13	A-7 Unidentified	-----------
52	19.17	A-9 Cyclohexanamine, N-cyclohexyl-N-methyl-	007560-83-0
53	19.28	A-7 Unidentified	-----------
54	19.45	A-5 Unidentified	-----------
55	19.65	A-2 Unidentified	-----------
56	19.71	A-10 Thiophene, 2-butyl-5-(2-methylpropyl)-	054845-35-1
57	20.36	A-6 A C8 alkyl phenol	-----------
58	20.49	A-3 Benzothiazole, 2-(methylthio)-	000615-22-5
59	20.62	A-6 Benzenamine, N-phenyl-	000122-39-4
60	20.92	A-10 Unidentified	-----------

TIRE CRUMB WATER BATCH 9A - continued.

Entry	R.T.	Conc.	CAS No.	Identity
61	21.07	A-7	-----------	Unidentified
62	21.13	A-3	-----------	Unidentified
63	21.39	A-300	000934-34-9	2(3H)-Benzothiazolone
64	21.84	A-6	-----------	An alkylated aromatic hydrocarbon
65	21.97	A-10	-----------	A linear alcohol ethoxylate
66	22.25	A-10	-----------	Unidentified
67	22.50	A-5	-----------	Unidentified
68	22.57	A-4	003622-84-2	Benzenesulfonamide, N-butyl-
69	22.63	A-5	-----------	Unidentified
70	22.80	A-7	-----------	An alkylated aromatic hydrocarbon
71	23.34	A-10	-----------	A biphenyl diol
72	23.61	A-3	-----------	Unidentified
73	23.84	A-4	000611-64-3	Acridine, 9-methyl- plus unidentified
74	23.91	A-4	-----------	Unidentified
75	24.04	A-10	-----------	Unidentified
76	24.16	A-10	-----------	Unidentified
77	24.25	A-9	006267-02-3	Acridine, 9,10-dihydro-9,9-dimethyl- (9C
78	24.67	A-4	-----------	Unidentified
79	24.74	A-4	-----------	Unidentified
80	24.96	A-40	-----------	A phenol
81	25.28	A-40	000102-77-2	Morpholine, 4-(2-benzothiazolylthio)-
82	25.43	A-4	-----------	Unidentified
83	25.54	A-20	002387-23-7	Urea, N,N'-dicyclohexyl-

TIRE CRUMB WATER BATCH 9A - continued.

Entry	R.T.	Conc. Identity	CAS No.
84	25.63	A-30 A phenol	-----------
85	26.03	A-6 Unidentified	-----------
86	26.23	A-4 Unidentified	-----------
87	26.31	A-4 Unidentified	-----------
88	26.59	A-10 A phenol	-----------
89	26.92	A-6 Unidentified	-----------
90	27.05	A-50 Unidentified	-----------
91	27.16	A-8 Unidentified	-----------
92	27.23	A-20 Guanidine, N,N'-bis(2-methylphenyl)- (9C	000097-39-2
93	27.45	A-5 Unidentified	-----------
94	28.42	A-90 A methyl naphthofurandione	-----------
95	28.49	A-30 Unidentified	-----------
96	28.76	A-4 Unidentified	-----------
97	28.87	A-20 Unidentified	-----------
98	29.03	A-10 Unidentified	-----------
99	29.30	A-300 Unidentified	-----------
100	29.46	A-6 Unidentified	-----------
101	29.80	A-20 Bis(2-ethylhexyl) phthalate	000117-81-7
102	30.14	A-8 Unidentified	-----------
103	30.18	A-7 Unidentified	-----------
104	30.32	A-60 Unidentified	-----------
105	30.63	A-10 Unidentified	-----------
106	31.08	A-6 Unidentified	-----------

TIRE CRUMB WATER BATCH 9A - continued.

Entry	R.T.	Conc. Identity	CAS No.
107	31.27	A-5 Unidentified	-----------
108	31.41	A-7 Unidentified	-----------
109	31.54	A-2 Unidentified	-----------
110	31.61	A-3 Unidentified	-----------
111	34.05	A-4 Unidentified	-----------

TIRE CRUMB WATER PH 2 - ACID FRACTION

(4-L aqueous extract at pH 2 of 100 grams of 30 to 40 mesh size tire granules)

Entry	R.T.	Conc. Identity	CAS No.
1	5.355	A-10 2-Pentanone, 4-methyl-	000108-10-1
2	7.909	A-8 An alcohol or ether	-----------
3	8.547	A-6 Unidentified	-----------
4	8.623	A-30 Cyclohexanone	000108-94-1
5	8.899	A-10 Ethanol, 2-butoxy-	000111-76-2
6	9.201	A-800 2,5-Cyclohexadiene-1,4-dione	000106-51-4
7	10.37	A-40 Aniline	000062-53-3
8	10.45	A-30 Phenol	000108-95-2
9	11.30	A-10 Unidentified	-----------
10	11.56	A-5 Unidentified	-----------
11	12.11	A-10 A hydrocarbon	-----------
12	12.19	A-40 A methyl-benzenamine	-----------
13	12.41	A-9 Benzyl alcohol, α,α-dimethyl-	000617-94-7
14	12.68	A-20 1-Propanamine, N-ethyl-N-methyl-	004458-32-6

TIRE CRUMB WATER PH 2 - ACID FRACTION - continued.

Entry	R.T.	Conc. / Identity	CAS No.
15	13.08	A-80 Hexanoic acid, 2-ethyl-	000149-57-5
16	13.14	A-20 Unidentified	-----------
17	13.94	A-20 Unidentified	-----------
18	13.98	A-8 Unidentified	-----------
19	14.28	A-10 3-cyclohexene-1-methanol, α,α,4-trimethyl-	000098-55-5
20	14.61	A-3 A nitrogen compound	-----------
21	14.91	A-600 Benzothiazole	000095-16-9
22	15.00	A-20 Unidentified	-----------
23	15.14	A-20 A tetrahydromethyl-thiophene	-----------
24	15.30	A-40 Formamide, N-cyclohexyl-	000766-93-8
25	15.41	A-20 Thiophene, 2-ethyltetrahydro-	001551-32-2
26	15.49	A-30 Unidentified	-----------
27	15.84	A-20 A butylphenol	-----------
28	16.55	A-7 Unidentified	-----------
29	16.67	A-7 Unidentified	-----------
30	16.82	A-20 Propanoic acid, 2-methyl-, 2,2-dimethyl-	074367-33-2
31	16.97	A-9 Unidentified	-----------
32	17.08	A-10 Propanoic acid, 2-methyl-3-hydroxy-2,4,4-trimethyl pentyl ester-	074367-34-3
33	17.20	A-50 Benzenamine, N-(2,2-dimethylpropyl)-N-methyl-	053927-61-0
34	17.26	A-6 Unidentified	-----------
35	17.47	A-6 A dihydroxy-methyl-benzaldehyde	-----------
36	17.57	A-6 Unidentified	-----------

TIRE CRUMB WATER PH 2 - ACID FRACTION - continued.

Entry	R.T.	Conc. / Identity	CAS No.
37	17.87	A-4 Unidentified	-----------
38	18.16	A-90 An aromatic nitrogen compound	-----------
39	18.43	A-500 1H-Isoindole-1,3(2H)-dione + Bicyclo[3.1.0]hexan-2-one, 1,5-bis(butyl)-3,3-dimethyl-	85-41-6/19377-95-8
40	18.69	A-20 Unidentified	-----------
41	19.00	A-10 Unidentified	-----------
42	19.14	A-10 Unidentified	-----------
43	19.51	A-3 Unidentified	-----------
44	19.59	A-30 A dibutylthiophene	-----------
45	20.04	A-10 Diethyl phthalate	000084-66-2
46	20.24	A-4 A phenol	-----------
47	20.50	A-9 Benzenamine, N-phenyl-	000122-39-4
48	20.83	A-9 Unidentified	-----------
49	20.93	A-10 Unidentified	-----------
50	20.99	A-3 Unidentified	-----------
51	21.11	A-200 2(3H)-Benzothiazolone	000934-34-9
52	21.72	A-8 Unidentified	-----------
53	22.11	A-10 A butyl-Indole	-----------
54	25.14	A-60 1,2-Benzisothiazole, 3-(4-morpholinyl)-	-----------
55	25.30	A-10 Unidentified	-----------
56	25.37	A-10 Urea, N,N'-dicyclohexyl-	002387-23-7
57	25.47	A-10 Unidentified	-----------
58	25.89	A-4 Unidentified	-----------

TIRE CRUMB WATER PH 2 - ACID FRACTION - continued.

Entry	R.T.	Conc. Identity	CAS No.
59	26.74	A-9 Unidentified	-----------
60	28.32	A-10 Unidentified	-----------
61	28.61	A-50 Unidentified	-----------
62	30.03	A-50 Unidentified	-----------
63	30.23	A-20 Unidentified	-----------
64	30.46	A-6 Unidentified	-----------
65	30.64	A-9 Unidentified	-----------
66	30.72	A-7 Unidentified	-----------
67	30.93	A-700 Unidentified	-----------
68	31.17	A-20 Unidentified	-----------
69	31.23	A-40 Unidentified	-----------
70	31.36	A-9 Unidentified	-----------
71	31.48	A-20 Unidentified	-----------
72	31.59	A-60 Unidentified	-----------
73	31.81	A-6 Unidentified	-----------
74	32.39	A-5 Unidentified	-----------
75	32.58	A-10 Unidentified	-----------
76	32.72	A-10 Unidentified	-----------
77	32.88	A-20 Unidentified	-----------
78	33.00	A-10 Unidentified	-----------
79	33.22	A-30 Unidentified	-----------
80	33.33	A-10 Unidentified	-----------
81	34.14	A-40 Unidentified	-----------

TIRE CRUMB WATER PH 2 - ACID FRACTION - continued.

Entry	R.T.	Conc. Identity	CAS No.
82	35.38	A-5 Unidentified	-----------
83	38.80	A-20 Unidentified	-----------
84	39.13	A-10 Unidentified	-----------
85	45.52	A-200 Unidentified	-----------
86	47.99	A-40 Unidentified	-----------

TIRE CRUMB WATER PH 2 - BASE-NEUTRAL FRACTION

(4-L aqueous extract at pH 2 of 100 grams of 30 to 40 mesh size tire granules)

Entry	R.T.	Conc. Identity	CAS No.
1	5.26	A-3 Unidentified	-----------
2	5.50	A-10000 An amine	-----------
3	5.94	A-200 Benzene, methyl-	000108-88-3
4	5.99	A-200 Piperazine, 1-methyl-	000109-01-3
5	6.34	A-200 Morpholine, 4-methyl-	000109-02-4
6	6.55	A-70 Unidentified	-----------
7	6.76	A-200 A nitrogen compound	-----------
8	7.70	A-2000 2-Hexanamine, 5-methyl-	028292-43-5
9	7.90	A-4000 Cyclohexanamine	000108-91-8
10	8.40	A-30 A nitrogen compound	-----------
11	8.46	A-50 Unidentified	-----------
12	8.63	A-40 Unidentified	-----------
13	8.91	A-200 A Hexanol	-----------
14	9.46	A-50 Unidentified	-----------

TIRE CRUMB WATER PH 2 - BASE-NEUTRAL FRACTION - continued.

Entry	R.T.	Conc. / Identity	CAS No.
15	9.64	A-90 Unidentified	-----------
16	10.08	A-60 A C-6 alkyl amine	-----------
17	10.66	A-40000 Aniline	000062-53-3
18	10.82	A-100 Unidentified	-----------
19	10.92	A-100 Unidentified	-----------
20	12.10	A-400 A methyl benzenamine	-----------
21	12.29	A-10000 A methyl benzenamine	-----------
22	12.70	A-100 An amine	-----------
23	12.99	A-70 A nitrogen compound	-----------
24	13.07	A-400 A nitrogen compound	-----------
25	13.19	A-90 A nitrogen compound	-----------
26	13.60	A-100 Benzenamine, N-(1-methylethyl)-	000768-52-5
27	13.89	A-100 A dimethyl aniline and unidentified	-----------
28	14.15	A-80 Ethanol, 2-(2-butoxyethoxy)-	000112-34-5
29	14.24	A-300 Unidentified	-----------
30	14.48	A-200 A chloro methyl pyridine	-----------
31	14.91	A-80 Benzothiazole	000095-16-9
32	14.95	A-200 A diethyl aniline	-----------
33	15.13	A-50 A nitrogen compound	-----------
34	15.22	A-80 Caprolactam	000105-60-2
35	15.30	A-300 Formamide, N-cyclohexyl-	000766-93-8
36	15.48	A-100 Isoquinoline	000119-65-3
37	15.83	A-100 Acetamide, N-cyclohexyl-	001124-53-4

TIRE CRUMB WATER PH 2 - BASE-NEUTRAL FRACTION - continued.

Entry	R.T.	Conc.	CAS No.	Identity
38	16.12	A-100	-----------	An amine
39	16.28	A-200	-----------	An amine
40	16.77	A-90	000054-11-5	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-
41	16.89	A-20	-----------	A nitrogen compound
42	16.95	A-70	-----------	A nitrogen compound
43	17.21	A-100	-----------	A C-6 alkyl aniline
44	17.28	A-40	-----------	A methyl quinoline
45	17.85	A-10000	000101-83-7	Cyclohexanamine, N-cyclohexyl-
46	18.02	A-90	-----------	Unidentified
47	18.18	A-500	-----------	A dimethyl quinoline
48	18.41	A-10000	-----------	A nitrogen compound
49	18.73	A-50	-----------	A nitrogen compound
50	18.77	A-60	-----------	A nitrogen compound
51	18.85	A-100	-----------	Unidentified
52	19.01	A-1000	007560-83-0	Cyclohexanamine, N-cyclohexyl-N-methyl-
53	19.33	A-300	-----------	Unidentified
54	19.78	A-30	-----------	Unidentified
55	20.27	A-200	-----------	Unidentified
56	20.58	A-90	-----------	Unidentified
57	20.64	A-50	-----------	Unidentified
58	20.84	A-1000	-----------	Unidentified
59	20.93	A-200	-----------	A nitrogen compound
60	21.02	A-100	016954-69-1	2-Benzothiazolamine, N-methyl-

TIRE CRUMB WATER PH 2 - BASE-NEUTRAL FRACTION - continued.

Entry	R.T.	Conc. Identity	CAS No.
61	21.14	A-90 Unidentified	-----------
62	21.46	A-60 Unidentified	-----------
63	21.85	A-50 Unidentified	-----------
64	21.97	A-40 Unidentified	-----------
65	22.11	A-600 Unidentified	-----------
66	23.70	A-70 Unidentified	-----------
67	24.32	A-20 1-methyl-2-Phenylbenzimidazole	002622-63-1
68	25.46	A-800 Unidentified	-----------
69	25.85	A-50 Unidentified	-----------
70	26.25	A-100 Unidentified	-----------
71	26.42	A-100 Unidentified	-----------
72	26.54	A-20 Unidentified	-----------
73	26.83	A-500 Unidentified	-----------
74	28.05	A-200 Unidentified	-----------
75	28.61	A-90 Unidentified	-----------
76	28.82	A-10 Unidentified	-----------
77	30.58	A-60 Unidentified	-----------
78	30.65	A-20 Unidentified	-----------
79	30.91	A-200 Unidentified	-----------
80	31.08	A-20 Unidentified	-----------
81	31.24	A-100 Unidentified	-----------
82	31.60	A-3000 Unidentified	-----------
83	31.75	A-10 Unidentified	-----------

TIRE CRUMB WATER PH 2 - BASE-NEUTRAL FRACTION - continued.

Entry	R.T.	Conc. Identity	CAS No.
84	31.89	A-30 Unidentified	-----------
85	32.61	A-60 Unidentified	-----------
86	32.88	A-100 Unidentified	-----------
87	33.30	A-100 Unidentified	-----------
88	34.12	A-60 Unidentified	-----------
89	34.94	A-100 Unidentified	-----------
90	35.04	A-30 Unidentified	-----------
91	38.60	A-30 Unidentified	-----------
92	48.00	A-200 Unidentified	-----------

TIRE CRUMB WATER PH 11

(4-L aqueous extract at pH 11 of 100 grams of 30 to 40 mesh size tire granules)

Entry	R.T.	Conc. Identity	CAS No.
1	6.144	A-1 Propanedioic acid, dimethyl-	000595-46-0
2	6.447	A-5 Morpholine, 4-methyl-	000109-02-4
3	6.644	A-2 A carboxylic acid	-----------
4	7.419	A-0.6 Unidentified	-----------
5	7.566	A-0.4 Unidentified	-----------
6	7.746	A-2 Ethanol, 2-(2-propenyloxy)-	000111-45-5
7	7.953	A-7 An alcohol	-----------
8	8.043	A-3 Butanoic acid, 3-methyl-	000503-74-2
9	8.191	A-2 An ester	-----------

TIRE CRUMB WATER PH 11 - continued.

Entry	R.T.	Conc. Identity	CAS No.
10	8.356	A-2 An amine	-----------
11	8.488	A-4 A hexylamine	-----------
12	8.666	A-20 Cyclohexanone	000108-94-1
13	8.727	A-20 A hexylamine	-----------
14	8.967	A-40 Ethanol, 2-butoxy-	000111-76-2
15	9.079	A-5 A hexylamine	-----------
16	9.241	A-5 Unidentified	-----------
17	9.462	A-2 An unsaturated hydrocarbon	-----------
18	9.672	A-6 An amine	-----------
19	9.908	A-0.7 Unidentified	-----------
20	10.01	A-1 A hydrocarbon + a C4-alkylbenzene	-----------
21	10.37	A-20 Aniline	000062-53-3
22	10.46	A-30 Phenol	000108-95-2
23	10.71	A-6 Urea, tetramethyl-	000632-22-4
24	10.83	A-30 A carboxylic acid	-----------
25	11.12	A-2 Unidentified	-----------
26	11.22	A-2 A carboxylic acid	-----------
27	11.37	A-7 Hexanol, 2-ethyl	000104-76-7
28	11.53	A-3 2,5-Furandione, 3,4-dimethyl-	000766-39-2
29	11.68	A-5 A hydroxy-benzaldehyde,	-----------
30	11.84	A-4 A methylphenol	-----------
31	12.09	A-4 Ethanone, 1-phenyl-	000098-86-2
32	12.20	A-8 A methyl-benzenamine	-----------

TIRE CRUMB WATER PH 11 - continued.

Entry	R.T.	Conc. Identity	CAS No.
33	12.37	A-3 A methylphenol	-----------
34	12.45	A-9 Benzenemethanol, α,α-dimethyl	000617-94-7
35	12.62	A-10 A carboxylic acid	-----------
36	12.76	A-3 A carboxylic acid	-----------
37	12.86	A-10 An amine	-----------
38	13.03	A-2 A carboxylic acid	-----------
39	13.15	A-20 Morpholine, 4-ethyl-	000100-74-3
40	13.24	A-5 An amine	-----------
41	13.84	A-300 A carboxylic acid	-----------
42	13.95	A-50 A linear alcohol ethoxylate	-----------
43	14.02	A-20 A linear alcohol ethoxylate	-----------
44	14.12	A-4 A methyl ester	-----------
45	14.19	A-1 Unidentified	-----------
46	14.27	A-20 Ethanol, 2-(2-butoxyethoxy)-	000112-34-5
47	14.42	A-200 A carboxylic acid + unidentified	-----------
48	14.72	A-20 A methyl ester	-----------
49	15.05	A-300 Benzothiazole	000095-16-9
50	15.24	A-400 A methyl ester	-----------
51	15.78	A-70 A methyl ester	-----------
52	15.89	A-70 A butyl-phenol	-----------
53	16.15	A-500 A methyl ester + unidentified	-----------
54	16.46	A-10 Formamide, N-phenyl-	000103-70-8
55	16.63	A-20 A methyl ester	-----------

TIRE CRUMB WATER PH 11 - continued.

Entry	R.T.	Conc. / Identity	CAS No.
56	16.68	A-40 A methyl ester	-----------
57	16.83	A-20 Unidentified	-----------
58	16.89	A-10 A methyl ester	-----------
59	16.98	A-10 A methyl ester	-----------
60	17.24	A-100 Decanoic acid	000334-48-5
61	17.52	A-4 A hydroxy-methoxy-benzaldehyde	000621-59-0
62	17.91	A-20 A methyl ester	-----------
63	18.19	A-20 A methyoxy-naphthalene	-----------
64	18.43	A-60 A linear alcohol ethoxylate	-----------
65	18.49	A-70 A C10-alkylphenol	-----------
66	19.62	A-50 A dibutyl-thiophene	-----------
67	19.82	A-200 A C12-carboxylic acid	-----------
68	20.08	A-10 Diethyl phthalate	000084-66-2
69	20.28	A-6 A dibutyl-phenol	-----------
70	20.39	A-8 Benzothiazole, 2-(methylthio)-	000615-22-5
71	20.52	A-10 Diphenylamine	000122-39-4
72	20.91	A-10 A carboxylic acid	-----------
73	21.02	A-3 Benzenamine, 2-methyl-N-phenyl-	001205-39-6
74	21.51	A-300 2(3H)-Benzothiazolone	000934-34-9
75	21.75	A-9 A C5-alkyl-naphthalene	-----------
76	21.93	A-30 A linear alcohol ethoxylate	-----------
77	22.06	A-40 A C14-carboxylic acid	-----------
78	22.17	A-10 Unidentified	-----------

TIRE CRUMB WATER PH 11 - continued.

Entry	R.T.	Conc. / Identity	CAS No.
79	22.26	A-20 A C10-alkyl-phenol	-----------
80	22.51	A-7 Benzenesulfonamide, N-butyl-	003622-84-2
81	22.71	A-9 A C5-alkyl-naphthalene	-----------
82	23.08	A-9 Unidentified	-----------
83	23.26	A-20 A biphenyl-diol	-----------
84	23.84	A-5 Unidentified	-----------
85	23.93	A-20 Unidentified	-----------
86	24.02	A-10 Unidentified	-----------
87	24.15	A-4 A dimethyl-dihydroacridine	-----------
88	24.20	A-6 A C16-carboxylic acid	-----------
89	24.59	A-90 2-Mercaptobenzothiazole	000149-30-4
90	24.86	A-50 A methylenebis-phenol	-----------
91	25.18	A-40 1,2-Benzisothiazole, 3-(4-morpholinyl)-	-----------
92	25.54	A-20 A methylenebis-phenol	-----------
93	26.52	A-7 Unidentified	-----------
94	27.12	A-70 An octahydrodimethyl-phenanthrenecarboxylic acid	-----------
95	27.73	A-300 A methyl ester of a C20-carboxylic acid	-----------
96	28.18	A-200 A resin acid	-----------
97	28.39	A-300 A resin acid	-----------
98	29.55	A-2000 A resin acid	-----------
99	30.61	A-100 Unidentified	-----------
100	31.33	A-10 Unidentified	-----------

TIRE CRUMB WATER PH 11 - continued.

Entry	R.T.	Conc.	CAS No.	Identity
101	31.52	A-7	-----------	Unidentified

TIRE CRUMB WATER PH 8

(4-L aqueous extract at pH 8 of 100 grams of 30 to 40 mesh size tire granules)

Entry	R.T.	Conc.	CAS No.	Identity
1	5.587	A-3	-----------	An amine
2	6.605	A-0.3	-----------	A halogenated compound
3	8.669	A-0.4	000108-94-1	Cyclohexanone
4	10.38	A-0.9	000062-53-3	Aniline
5	12.35	A-0.8	000637-88-7	1,4-Cyclohexanedione
6	13.12	A-1	-----------	A carboxylic acid
7	13.26	A-0.3	-----------	A terpene
8	14.24	A-0.5	000091-20-3	Naphthalene
9	14.33	A-0.5	-----------	A cyclic ketone
10	14.91	A-20	000095-16-9	Benzothiazole
11	15.41	A-1	-----------	A carboxylic acid
12	15.85	A-1	-----------	A butyl-phenol
13	16.29	A-0.3	-----------	A dimethyl-naphthalene
14	16.68	A-2	-----------	Unidentified
15	16.82	A-2	-----------	A carboxylic acid ester
16	17.09	A-0.7	-----------	A carboxylic acid ester
17	17.21	A-0.2	-----------	Unidentified
18	17.59	A-1	-----------	Unidentified

TIRE CRUMB WATER PH 8 - continued.

Entry	R.T.	Conc. Identity	CAS No.
19	18.18	A-1 A methoxy-naphthalene	-----------
20	18.45	A-20 A C10-alkylphenol	-----------
21	19.03	A-6 Unidentified	-----------
22	19.15	A-1 Unidentified	-----------
23	19.30	A-3 Unidentified	-----------
24	19.34	A-2 Unidentified	-----------
25	19.45	A-4 Unidentified	-----------
26	19.59	A-2 A dibutyl-thiophene	-----------
27	20.09	A-0.8 A carboxylic acid ester	-----------
28	20.25	A-2 A C8-alkylphenol	-----------
29	20.40	A-20 Benzothiazole, 2-(methylthio)-	000615-22-5
30	20.73	A-0.6 Unidentified	-----------
31	21.00	A-0.9 Benzenamine, 2-methyl-N-phenyl-	001205-39-6
32	21.10	A-4 2(3H)-Benzothiazolone	000934-34-9
33	21.46	A-0.4 Unidentified	-----------
34	21.56	A-0.7 Unidentified	-----------
35	21.72	A-3 A C5-alkyl-naphthalene	-----------
36	22.09	A-0.8 Unidentified	-----------
37	22.24	A-1 Unidentified	-----------
38	22.67	A-2 A C5-alkyl-naphthalene	-----------
39	24.05	A-2 Unidentified	-----------
40	24.13	A-2 A dihydro-dimethyl-acridine	-----------
41	25.14	A-1 1,2-Benzisothiazole, 3-(4-morpholinyl)-	-----------

TIRE CRUMB WATER PH 8 - continued.

Entry	R.T.	Conc. / Identity	CAS No.
42	25.31	A-0.6 Unidentified	-----------
43	25.47	A-0.7 Unidentified	-----------
44	25.90	A-0.6 Unidentified	-----------
45	26.32	A-1 Unidentified	-----------
46	26.84	A-5 Unidentified	-----------
47	27.08	A-1 Unidentified	-----------
48	27.24	A-0.4 1-Naphthalenamine, N-phenyl-	000090-30-2
49	28.16	A-3 Unidentified	-----------
50	28.63	A-1 Unidentified	-----------
51	28.69	A-5 Unidentified	-----------
52	30.04	A-0.9 Unidentified	-----------
53	30.49	A-1 Unidentified	-----------
54	30.94	A-2 A methyl ester of a resin acid	-----------
55	31.24	A-2 Unidentified	-----------
56	31.46	A-2 Unidentified	-----------
57	31.59	A-1 Unidentified	-----------
58	32.24	A-0.8 An alkane	-----------
59	33.05	A-2 An alkane	-----------
60	33.90	A-2 An alkane	-----------
61	34.85	A-1 An alkane	-----------
62	35.94	A-1 An alkane	-----------
63	36.52	A-0.9 Unidentified	-----------
64-66	37-40	A-1 three alkanes	-----------

APPENDIX G

GC/MS ANALYSIS FOR VOLATILE ORGANIC COMPOUNDS: TIRE WATER

Volatile organic compounds were analyzed by a purge-and-trap system followed by gas chromatography/(full scan) mass spectrometry (GC/(FS)MS). The concentrations are approximate and were calculated relative to the internal standard d_{10}-ethylbenzene. No volatile organic compounds were detected in tire water batch 4.

APPENDIX H

LC/MS ANALYSIS FOR EXTRACTABLE ORGANIC COMPOUNDS: TIRE WATER

Extractable organic compounds were determined in the following manner: The extracts were divided into base, neutral and acid fractions and were analyzed by reverse phase liquid chromatography/(particle beam) mass spectrometry. Most of the compounds detected in tire water batch 5 were also detected in the GC/MS scans except for an alkylated phenol that was probably from an antioxidant mixture from the tire.

APPENDIX I

SCANNING ELECTRON MICROSCOPY: TIRE CRUMB MATERIAL

A sample of tire crumb material was fractionated by separating the white and black particles found in the mixture. Examination of the particles by scanning electron microscopy indicated that the white material was primarily titanium with lesser amounts of silicon and aluminium. This was probably titanium oxide whitewall paint. Examination of samples of the black particles showed that it was a heterogeneous mixture composed primarily of silicon, sulphur and zinc with lesser amounts of calcium and iron. An "unresolved envelope" of organic compounds was noted in all samples.

APPENDIX J

UV/VIS SPECTROMETRY: TIRE WATER AND TIRE CRUMB WATER

A sample of tire water (batch 10) was scanned in the ultraviolet (UV) and visible (VIS) light ranges. No significant light absorbance was found. Four samples of tire crumb water (batch 5) were scanned in the UV range. No significant light absorbance was found in the following samples: baseline, pH 3, pH 11 or spiked with a 1% (wt/vol) phenol solution

APPENDIX K

FTIR ANALYSIS: TIRE CRUMB MATERIAL

A sample of tire crumb material was Soxhlet-extracted with dichloromethane. This gave a significant quantity (96,450 ppm) of extract, a viscous dark-brown liquid. Fourier Transform Infrared (FTIR) spectra on this liquid indicated it contained a mixture of paraffinic, naphthenic and aromatic hydrocarbons with a minor quantity of carbonyl compounds (esters and possibly acids). The spectra resembled those obtained from lubricant oils.

APPENDIX L

CHEMICAL ANALYSES FOR TOTAL UNFILTERED REACTIVE PHENOLS[1]: TIRE WATER, SURFACE WATER AND TIRE CRUMB WATER

Phenols (ug\L)	Tire water batch	Tire submersion time (days)
0.6	4	5
2.6	4	8
2.2	4	12
3.6	5a	7
2.6	5a	9
2.8	5a	12
2.8	5a	15
2.2	5a	16[1]
2.0	5a	16[2]
2.0	5a	16[3]
4.4	5b	26
2.4	6	8
3.0	6	13
nd	7	1
nd	7	6
1.8	7	13
nd	10	15
1.6	13c	13
Ambient tire water (field samples)		
nd	Lake Erie reef	approx. 1 year
nd	trench site	approx. 3 years
Tire crumb water		
13	na	pH 8
65	na	pH 11
220	na	pH 2

[1] Phenols with alkyl, aryl, nitro, benzoyl, nitroso or aldehyde *para*-substituents are not well-measured by this test, so the reported concentrations are minima. Phenols were generally nondetectable (<0.2 ug/L) or very low in the control water samples. nd = not detected; [2] sample stored 10 days at 20°C in the dark; [3] sample stored 10 days at 20°C under light;[4] sample stored 10 days at 15°C under light

APPENDIX M

ACUTE LETHALITY TO RAINBOW TROUT OF FLOW-THROUGH TIRE WATER: TIME TO 50% MORTALITY (LT50) IN THE SINGLE CONCENTRATION TESTS[1]

Lake Erie reef tires			trench site tires			scrap tires group C		
Flow time	Flow rate	LT50	Flow time	Flow rate	LT50	Flow time	Flow rate	LT50
7	1	30.9	5	0.5	NL	15	0.5	42.5
11	1	32.2	7	0.5	NL	21	1	NL
21	1	36.1	8	1	NL	22	1.5	NL
29	2	>96	9	1	NL	36	1	NL
31	2	NL	12	1	NL	39	0.5	NL
36	2	NL	14	0.5	NL	42	0.5	>96
43	1.5	NL	16	0.5	NL			
58	1.5	NL	20	0.5	95			
65	1	NL	21	0.25	73			
71	0.5	47.8	22	0.25	79.5			
74	0.5	50	23	0.25	>96			
78	1	NL	26	0.25	53.8			
84	1	NL	28	0.25	>96			
			32	0.25	>96			
			39	0.16	34			
			68	0.38	NL			
			69	0.36	NL			
			78	0.34	NL			

1 95 % confidence limits could not be calculated because partial mortality (> 0 to < 100 %) was not observed.

Table legend

Flow time cumulative days
Flow rate L/min
LT50 hours
NL nonlethal, no fish died
>96 % mortality >0 and <50

APPENDIX N

ACUTE LETHALITY TO RAINBOW TROUT OF FLOW-THROUGH TIRE WATER: PERCENTAGE MORTALITY IN THE DILUTION SERIES TESTS

The tires were collected from the artifical reef in Lake Erie.

Test 38: 1.0 L/min flow for 11 days

Concentration	**Exposure time**			
% vol/vol	**24 hours**	**48 hours**	**72 hours**	**96 hours**
100	0	100	100	100
80	0	80	100	100
65	0	40	70	80
40	0	0	30	50
30	0	0	0	0
0	0	0	0	0
LC50	**NL**	**68**	**50**	**45**
95 % C.L.s		56-76	43-59	38-53

Test 40: 1.0 L/min flow for 21 days

Concentration	**Exposure time**			
% vol/vol	**24 hours**	**48 hours**	**72 hours**	**96 hours**
100	0	80	90	90
80	0	60	70	80
65	0	0	30	30
40	0	0	0	0
30	0	0	0	0
20	0	0	0	0
0	0	0	0	0
LC50	**NL**	**83**	**73**	**71**
95 % C.L.s		75-93	61-82	60-80

Test 41: 2.0 L/min flow for 29 days

Concentration	**Exposure time**		
% vol/vol	**24 hours**	**48 hours**	**72 hours**
100	0	0	30
80	0	0	10
65	0	0	0
40	0	0	0
0	0	0	0
LC50	**NL**	**NL**	**>100**

Test 42: 2.0 L/min flow for 31 days

Concentration	**Exposure time**			
% vol/vol	**24 hours**	**48 hours**	**72 hours**	**96 hours**
100	0	0	0	0
80	0	0	0	0
65	0	0	0	0
0	0	0	0	0
LC50	**NL**	**NL**	**NL**	**NL**

Test 43: 2.0 L/min flow for 36 days

Concentration	**Exposure time**			
% vol/vol	**24 hours**	**48 hours**	**72 hours**	**96 hours**
100	0	0	0	0
80	0	0	0	0
65	0	0	0	0
0	0	0	0	0
LC50	**NL**	**NL**	**NL**	**NL**

APPENDIX O

ACUTE LETHALITY TO RAINBOW TROUT OF STATIC TIRE WATER: PERCENTAGE MORTALITY IN THE DILUTION SERIES TESTS

Test 17: batch 7

Concentration % vol/vol	Exposure time 24 hours	48 hours	72 hours	96 hours
100	100	100	100	100
65	17	83	83	83
40	0	33	33	33
30	0	0	0	0
0	0	0	0	0
LC50	**75**	**48**	**48**	**48**
95 % C.L.s	**65-86**	**40-59**	**40-59**	**40-59**

Test 18: batch 7 (6d-old)

Concentration % vol/vol	Exposure time 24 hours	48 hours	72 hours	96 hours
100	100	100	100	100
65	20	90	100	100
40	0	20	60	60
0	0	0	0	0
LC50	**75**	**53**	**43**	**43**
95 % C.L.s	**68-82**	**45-61**	**N.C.**	**N.C.**

Test 19: batch 8

Concentration % vol/vol	Exposure time 24 hours	48 hours	72 hours
100	100	100	100
65	80	100	100
40	10	10	20
0	0	0	0
LC50	**56**	**47**	**47**
95 % C.L.s	**68-70**	**N.C.**	**N.C.**

Test 21: batch 9

Concentration % vol/vol	Exposure time 24 hours	48 hours	72 hours	96 hours
100	90	100	100	100
65	50	100	100	100
40	0	50	80	80
30	0	0	10	10
0	0	0	0	0
LC50	**68**	**42**	**36**	**36**
95 % C.L.s	**56-82**	**37-48**	**32-40**	**32-40**

Test 29: batch 12 (8d-old)

Concentration % vol/vol	Exposure time 24 hours	48 hours	72 hours	96 hours
100	90	100	100	100
65	20	80	100	100
40	0	80	100	100
30	0	60	90	90
0	0	0	0	0
LC50	**77**	**25**	**<30**	**<30**
95 % C.L.s	**65-92**	**N.C.**	**N.C.**	**N.C.**

Test 29: batch 12 (8d-old), distilled dilution water

Concentration % vol/vol	Exposure time 24 hours	48 hours	72 hours	96 hours
100	90	100	100	100
65	30	90	90	90
40	0	30	50	80
30	0	0	0	0
0	0	0	0	20
LC50	**74**	**51**	**44**	**44**
95 % C.L.s	**61-89**	**42-63**	**37-53**	**36-51**

Test 32: batch 13c

Concentration % vol/vol	Exposure time 24 hours	48 hours	72 hours	96 hours
100	100	100	100	100
80	80	100	100	100
65	50	100	100	100
40	0	10	60	80
30	0	0	30	30
20	0	0	10	10
0	0	0	0	0
LC50	**66**	**51**	**35**	**32**
95 % C.L.s	**53-74**	**N.C.**	**28-43**	**27-39**

Test 34: batch 13c (14d-old)

Concentration % vol/vol	Exposure time 24 hours	48 hours	72 hours	96 hours
80	0	70	100	100
65	0	50	80	90
40	0	0	0	0
0	0	0	0	0
LC50	**>80**	**65**	**52**	**51**
95 % C.L.s	**N.A.**	**55-81**	**47-57**	**N.A.**

Test 34: batch 13c (14d-old), distilled dilution water

Concentration % vol/vol	Exposure time 24 hours	48 hours	72 hours	96 hours
80	0	70	100	100
65	0	60	90	90
40	0	0	10	20
20	0	0	0	0
0	0	0	0	10
LC50	**> 80**	**63**	**49**	**47**
95 % C.L.s	**N.A.**	**53-78**	**42-56**	**40-54**

Test 35: batch 14

Concentration % vol/vol	Exposure time 24 hours	48 hours	72 hours	96 hours
100	100	100	100	100
80	100	100	100	100
65	60	100	100	100
40	0	0	50	60
30	0	0	0	0
0	0	0	0	0
LC50	**59**	**51**	**42**	**40**
95 % C.L.s	**53-65**	**40-65**	**37-48**	**36-46**

Test 36: batch 14 (20d-old)

Concentration % vol/vol	Exposure time 24 hours	48 hours	72 hours	96 hours
100	50	100	100	100
80	30	100	100	100
65	0	100	100	100
40	0	30	50	50
30	0	0	30	30
20	0	0	0	0
0	0	0	0	0
LC50	**> 96**	**45**	**37**	**37**
95 % C.L.s	**N.A.**	**41-51**	**32-44**	**32-44**

Test 46: batch 15 (3d-soak of tires)

Concentration	**Exposure time**			
% vol/vol	**24 hours**	**48 hours**	**72 hours**	**96 hours**
100	0	100	100	100
80	0	30	60	60
65	0	10	10	10
40	0	0	0	0
0	0	0	0	0
LC50	**NL**	**81**	**77**	**77**
95 % C.L.s		74-89	70-84	70-84

Test 66: Tire water batch 16b (3-day soak)

Concentration	**Exposure time**			
% vol/vol	**24 hours**	**48 hours**	**72 hours**	**96 hours**
100	100	100	100	100
80	70	100	100	100
65	60	100	100	100
40	10	30	30	40
30	0	10	10	50
20	0	0	0	0
0	0	0	0	0
LC50	**62**	**42**	**42**	**36**
95 % C.L.s	**52-72**	**37-51**	**37-51**	**30-44**

Test 67: Tire water batch 16c (fresh sample)

Concentration	**Exposure time**			
% vol/vol	**24 hours**	**48 hours**	**72 hours**	**96 hours**
65	100	100	100	100
40	0	100	100	100
30	0	80	100	100
20	0	20	40	40
10	0	0	0	0
0	0	0	0	0
LC50	**51**	**24**	**27**	**27**
95 % C.L.s	**N.C.**	**20-28**	**22-34**	**22-34**

APPENDIX P

ACUTE LETHALITY TO RAINBOW TROUT OF STATIC TIRE WATER SUBJECTED TO TOXICITY REDUCTION TREATMENTS

Percentages listed under *sample treatment* refer to tire water samples diluted to different volumetric ratios with dechlorinated municipal tap water. Distilled water was used as the diluent in some tests as indicated by *distilled diluent*. *Baseline* means the undiluted and untreated sample of tire water (TW) tested for toxicity in comparison to the corresponding treated samples. When toxicity testing occurred immediately after collecting the sample, the sample age (*age*) is given as 1 day. Where samples were stored for later treatment and testing, age refers to the storage time. Samples were stored in filled and sealed plastic-lined 20-L buckets at 15 °C in the dark unless indicated.

TW#	Tst #	Sample treatment	age (d)	LT50 (h)	95% C.L.'s
4	8	baseline	1	NL	N.A.
5a	9	baseline	1	4-20	N.A.
		pre-tested for 24h	1	23	N.A.
	10	stored @ 20°C	10	26.7	23.4-31.6
		stored @ 20°C under light	10	31.9	28.8-35.9
		stored @ 20°C under light in a glass container	10	33.2	28.6-42.9
		stored	10	24.6	21.6-34.2
	11	stored	26	18.7	7.7-21.2
5b	10	baseline	1	20.1	12.0-24.6
6a	11	baseline	1	2-18	N.A.
6b	12	baseline	1	2-17	N.A.
		pre-tested for 24h	1	15.1	N.A.
		pre-aerated for 24h	1	15.9	14.2-17.8
	13	stored	8	21.9	19.7-25.0
	14	stored	14	19.0	13.9-21.2
	15	stored	28	25.5	23.2-29.7
	16	stored	35	27.5	25.2-32.0
7	16	baseline	1	2-18	N.A.
	17	baseline	1	16.3	13.3-20.1

TW#	Tst #	Sample treatment	age (d)	LT50 (h)	95% C.L.'s
		65 %	1	30.8	25.1-56.3
		40 %	1	>96	N.A.
		30 %	1	NL	N.A.
	18	baseline	6	5-22	N.A.
		65 %	6	32.7	29.0-37.1
		46 %	6	71.6	59.2-100
	19	stored	13	20.5	13.5-24.6
		stored @ 20°C under light	13	20.8	12.7-24.0
8	19	baseline	1	6-19	N.A.
		65 %	1	17.9	8.7-21.4
		40 %	1	>72	N.A.
	20	stored	7	16.9	13.6-21.0
	21	stored	14	22.4	18.9-23.9
	22	stored	30	25.9	23.2-84.6
9	21	baseline	1	6-21	N.A.
		65 %	1	23.9	19.7-27.0
		40 %	1	51.7	41.3-62.4
		30 %	1	>96	N.A.
	22	stored	16	36.6	28.5-46.9
	23	stored	22	18.1	15.0-22.0
10	22	baseline	1	21.1	19.6-26.0
	23	stored,100 g carbon added	6	NL	N.A.
		stored,200 g carbon added	6	NL	N.A.
		stored	6	16.2	12.8-20.6
	24	stored, 50 g carbon added	12	NL	N.A.
	26	stored	20	26.1	23.2-28.4
	26	pre-aerated for 24h	20	30.1	27.9-39.3
11	24	baseline	1	1-17	N.A.
	25	baseline	1	1-17	N.A.
		65 %	1	28.1	25.6-30.9

TW#	Tst #	Sample treatment	age (d)	LT50 (h)	95% C.L.'s
		560 ppb $HgCl_2$ added to 100 % tire water	1	1-17	N.A.
		560 ppb $HgCl_2$ added to 65 % tire water	1	1-17	N.A.
		stored, 25 g carbon added	1	96	N.A.
	26	stored	7	2-18	N.A.
		pre-extracted with CH_2Cl_2 in separatory funnel	7	NL	N.A.
		tire water in separatory funnel without CH_2Cl_2	7	>96	N.A.
	28	stored	16	17.8	11.6-27.2
		steam-distilled	16	39.7	N.A.
	29	stored	20	14.9	9.2-24.1
12	27	baseline replicate A	1	4.5-21.5	N.A.
		baseline replicate B	1	4.5-21.5	N.A.
		65 %	1	31.9	26.9-40.8
		65 % - distilled diluent	1	4.5-21.5	N.A.
		stored, 50 g carbon added	1	NL	N.A.
	29	baseline	8	2.5-22	N.A.
		65 %	8	26.1	18.4-32.2
		65 % - distilled diluent	8	26.8	17.5-34.2
		40 %	8	42.6	34.9-46.7
		40 % - distilled diluent	8	68.9	56.7-93.6
		30 %	8	47.5	40.4-53.7
		30 % - distilled diluent	8	NL	N.A.
	30	baseline	8	11.9	5.8-24.3
		tire water in separatory funnel without CH_2Cl_2	8	29.2	N.A.
		pre-extracted with CH_2Cl_2 in separatory funnel	8	26-46	N.A.
13a	28	baseline	1	20.7	13.4-32.1
13b	30	baseline	1	0.5-17.5	N.A.

TW#	Tst #	Sample treatment	age (d)	LT50 (h)	95% C.L.'s
13c	31	baseline	1	19.50	13.5-28.2
	32	baseline	1	0.5-17	N.A.
		80 %	1	17.9	9.3-20.8
		65 %	1	22.4	20.0-26.7
		40 %	1	68.3	60.0-77.4
		30 %	1	> 96	N.A.
		20 %	1	> 96	N.A.
	33	stored	7	> 96	N.A.
		tire water in separatory funnel without CH_2Cl_2	7	NL	N.A.
		pre-extracted with CH_2Cl_2 in separatory funnel	7	NL	N.A.
	34	80 %	14	38.7	31.4-44.0
		80 % - distilled diluent	14	38.7	31.4-44.0
		65 %	14	47.8	39.2-57.3
		65 % - distilled diluent	14	43.0	35.5-50.1
		40 %	14	NL	N.A.
		40 % - distilled diluent	14	> 96	N.A.
		20 %	14	NL	N.A.
		20 % - distilled diluent	14	NL	N.A.
14	35	baseline	1	< 16.5	N.A.
		80 %	1	< 16.5	N.A.
		65 %	1	21.6	17.3-27.9
		40 %	1	67.0	N.A.
		30 %	1	NL	N.A.
		20 %	1	NL	N.A.
		10 %	1	NL	N.A.
	39	baseline	20	22.96	16.5-25.4
		80 %	20	30.35	26.6-37.3
		65 %	20	35.79	31.9-40.3
		40 %	20	93.85	N.A.

TW#	Tst #	Sample treatment	age (d)	LT50 (h)	95% C.L.'s
		30 %	20	>96	N.A.
		20 %	20	NL	N.A.
	38	ethanol extract of carbon used to detoxify tire water	1	>100	N.A.
		ethanol extract of carbon used in control water	1	NL	N.A.
15	46	baseline	1	21-49	N.A.
		80 %	1	67.8	52.8-100
		65 %	1	>96	N.A.
		40 %	1	>96	N.A.
		30 %	1	>96	N.A.
		20 %	1	>96	N.A.
16a	65	baseline	1	5-22	N.A.
16b	66	baseline	1	2-27	N.A.
		80 %	1	13.8	7.7-24.7
		65 %	1	16.8	8.9-31.6
		40 %	1	> 96	N.A.
		30 %	1	96	N.A.
		20 %	1	> 96	N.A.
		10 %	1	> 96	N.A.
16c	67	baseline	1	7-24	N.A.
		80 %	1	7-24	N.A.
		65 %	1	7-24	N.A.
		40 %	1	24-48	N.A.
		30 %	1	24-48	N.A.
		20 %	1	> 96	N.A.
		10 %	1	> 96	N.A.
17a	68	baseline	1	53	45.5-61.8
17b	69	baseline	1	14.8	8.1-26.9

APPENDIX Q

ACUTE LETHALITY TO *DAPHNIA MAGNA* OF TIRE CRUMB WATER SUBJECTED TO TOXICITY REDUCTION TREATMENTS

Tire crumb water batch#/description	24h-LC50 (% v/v)	95 % C.L.s	48h-LC50 (% v/v)	95 % C.L.s
3	89	61-100	19	13-26
6	79	48-100	23	18-28
7	> 100		50	35-73
8 filtered	> 100		100	
8 unfiltered	50-100		50	35-70
9A	> 100		56	39-88
9C	> 100		46	34-61
9D	> 100		35	27-46
9E filtered	> 100		50-100	
9E unfiltered	> 100		54	41-73
9F	> 100		74	59-92
9G	> 100		> 100	
9H	21	12-32	15	11-20
9I	81	66-100	77	64-93
9J	> 100		88	68-10
solvent-extracted crumb	> 100		> 100	
"fresh" tire crumb	91	72-100	85	69-100
pH 2	5	1-11	< 5	
pH 11	34	26-43	24	21-28
pH baseline	100	na	78	62-100
pH 2	9	4-13	< 5	
pH 11	24	18-32	15-30	
pH baseline	74	61-89	74	61-89
pH 4	> 100		60-100	
pH 10	49	na	42	na
filtered	> 100		59	46-73
unfiltered	> 100		45	32-61

APPENDIX R

ACUTE LETHALITY TO *DAPHNIA MAGNA* OF TIRE CRUMB WATER TREATED WITH EDTA

The EDTA stock concentration was 67.5 g/L. 0.2 mL of stock in a 50 mL test chamber gives 0.27 g/L which was the 48h-LC50 using the dilution water. Each of the six volumes of EDTA shown below was added to 50 mL of tire crumb water in a separate test chamber. The *baseline* was tire crumb water only (without EDTA) and the *control* was dilution water only. The experiment was conducted three times (replicates A, B and C).

	Number dead out of 12 animals per treatment					
EDTA volume (mL)	**Replicate A**		**Replicate B**		**Replicate C**	
	Exposure time (h)		**Exposure time (h)**		**Exposure time (h)**	
	24	**48**	**24**	**48**	**24**	**48**
0.4	12	12	12	12	11	12
0.2	12	12	12	12	11	12
0.1	6	10	4	11	10	12
0.050	7	9	2	7	9	10
0.025	5	8	2	6	8	8
0.012	4	12	0	3	7	7
baseline	12	12	12	12	12	12
control	0	1	0	1	1	1

APPENDIX S

ACUTE LETHALITY TO *DAPHNIA MAGNA* OF TIRE CRUMB WATER SUBJECTED TO SOLID PHASE EXTRACTIONS

Water samples were passed through a 3-mL C_{18} column and tested for toxicity before and after the column treatment. The columns were eluted (solvent-washed) with methanol, and the eluate was added to dilution water for toxicity testing. Four replicate experiments were conducted.

Repl.	**Sample**	**Treatment**	**% Mortality**
A	tire crumb water	before SPE	94 %
		after SPE	92 %
		methanol	nonlethal
	dilution water	before SPE	6 %
		after SPE	17 %
		methanol	nonlethal
B	tire crumb water	before SPE	92 %
		after SPE	100 %
		methanol	nonlethal
	dilution water	before SPE	nonlethal
		after SPE	nonlethal
		methanol	nonlethal
C	tire crumb water	before SPE	83 %
		after SPE	67 %
		methanol	nonlethal
	dilution water	before SPE	nonlethal
		after SPE	17 %
		methanol	nonlethal
D	tire crumb water	before SPE	92 %
		after SPE	17 %
	dilution water	before SPE	nonlethal
		after SPE	nonlethal

APPENDIX T
CHRONIC TOXICITY TO *CERIODAPHNIA DUBIA* OF TIRE CRUMB WATER

Tire crumb water batch 9b

Concentration (% vol/vol)	**% mortality**	**% inhibition of reproduction**
25	100	100
15	10	83
10	0	25
5	0	0
0	0	n = 20.75 *1*
LC50 or EC50 (95% C.L.s)	**19 (17-20)**	**12 (9-14)**

Tire crumb water batch 9c

Concentration (% vol/vol)	**% mortality**	**% inhibition of reproduction**
25	90	100
15	100	68
10	40	0
5	10	0
0	0	n = 27.85
LC50 or EC50 (95 % C.L.s)	**9 (7-12)**	**14 (12-16)**

Tire crumb water batch 9h

Concentration (% vol/vol)	**% mortality**	**% inhibition of reproduction**
25	100	100
15	60	95
10	30	67
5	20	26
0	10	n = 19.3
LC50 or EC50 (95 % C.L.s)	**11 (8-15)**	**7 (5-10)**

Tire crumb water batch 9m

Concentration (% vol/vol)	% mortality	% inhibition of reproduction
25	90	100
15	40	49
10	20	22
5	40	13
0	0	n = 12.4
LC50 or EC50 (95 % C.L.s)	**15 (12-21)**	**13 (9-17)**

Tire crumb water batch 7 - unfiltered

concentration (% vol/vol)	% mortality	% inhibition of reproduction
25	100	100
15	100	100
10	50	44
5	30	0
0	5	n = 31.0
LC50 or EC50 (95 % C.L.s)	**8 (5-10)**	**12 (10-15)**

Tire crumb water batch 7 - filtered

concentration (% vol/vol)	% mortality	% inhibition of reproduction
25	100	100
15	40	86
10	30	39
5	0	15
0	20	n = 20.75
LC50 or EC50 (95 % C.L.s)	**13 (11-17)**	**10 (7-12)**

1 The average number of young per female control animal is shown as "(n =)". The inhibition of reproduction was a percentage of this.

APPENDIX U

ACUTE LETHALITY TO TROUT OF SOLVENT-EXTRACTS OF TIRE CRUMB

extract and concentration	**LT50 (95% C.L.'s) (h)**
"tire oil"[1] (mg/L)	
43	4-24
solvent control	nonlethal
50	7-24
25	21
12.5	nonlethal
6	nonlethal
solvent control	nonlethal
methanol extract[2] (mL/L)	
5	35 (13-50)
solvent control	nonlethal
5	7-24
solvent control	nonlethal

[1] A dark brown viscous liquid ("tire oil") was soxhlet-extracted from tire crumb with dichloromethane. It was a mixture of paraffinic, naphthenic and aromatic hydrocarbons resembling lubricant oils. Dichloromethane was used to dissolve the tire oil in water. The solutions were pre-aerated for 24 hours to purge the solvent before adding the fish. The solvent controls had the same amount of carrier solvent as used for the highest test concentrations. Reported concentrations are nominal.

[2] 10 grams of tire crumb were stirred for 24 hours in 150 mL of methanol. The mixture was left quiescent for 24 hours so the rubber particles could "settle out", then 100 mL of the methanol was added to 20L of water for a trout test. The solvent controls had the same amount of carrier solvent as used for the highest test concentrations. Reported concentrations are nominal.