Bull Environ Contam Toxicol (2007) 79:114–119
DOI 10.1007/s00128-007-9145-3

Toxic Components Leaching from Tire Rubber

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Received: 9 January 2007 / Accepted: 16 March 2007 / Published online: 3 May 2007

Tire rubber is a complex mixture of a variety of chemicals, e.g., rubber polymers, carbon blacks, silicas, process and extender oils, vulcanization chemicals, and chemical antidegradents (Barbin and Rodgers, 1994). Leachates of tire rubber are toxic to a range of aquatic organisms; see for example the review by Evans (1997). Attempts made so far to identify the components causing the toxicity of rubber leachates have focused on chemical fractionation and characterization followed by toxicity testing of different fractions. Toxicity identification evaluations of leachates from tire plugs leached in deionized water have indicated that zinc was the main cause of toxicity to *Ceriodaphnia dubia* (Nelson et al., 1994). Microtox toxicity was caused by several organic compounds spread through a range of polarities (Anthony et al., 1995). A toxicity identification evaluation on leachates obtained from tire wear material leached in reconstituted water (pH 8, hardness 250 mg/L as $CaC0_3$), identified nonpolar organic compounds as the main cause of toxicity to *Daphnia magna* (Wik and Dave, 2006). In this previous study, we also found large differences in toxicity between different tires, which implies that the environmental impact of tire wear can be effectively reduced through rubber formulation if the most toxic components are identified.

The aim of this study was to identify the components in tire rubber that are toxic to *Daphnia magna*, using a novel approach. Rubber formulations containing different additives were produced, and water leachates from the rubber samples were prepared and tested for toxicity using a standardized toxicity test. The main objective of this study was therefore to assess the toxicity to *Daphnia magna* of different rubber formulations in order to identify the most toxic tire leachate components.

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Materials and Methods

All rubber samples were prepared as sheets by a tire manufacturer. A typical summer tire tread compound formula containing a minimum of components needed to make cured (vulcanized) rubber was prepared as a reference sample (sample 1). Twenty-one samples were then prepared by adding different additives to the reference formulation. Different process and extender oils were added to five samples (samples 2–6), different types of antidegradents (antioxidants and antiozonants) to six samples (samples 7–12), different types of vulcanization accelerators to six samples (samples 13–18), and different fillers/ reinforcing agents to four samples (samples 19–22). Table 1 shows the formulations of the different rubber samples. The rubber sheets were mailed to our laboratory immediately after preparation, leachates of the samples were prepared and toxicity tests performed in six test runs (samples 2–5, 6–9, 10–12, 13–15, 16–18, and 19–22). Rubber from each sample was cut into pieces of approximately $2 \times 2 \times 2$ mm and various amounts of the rubber were then placed in 50-mL Petri dishes and diluted with reconstituted water according to ISO (1996), with a hardness of 250 mg/L as $CaCO_3$ and pH 8.0, to give dilution series with six concentrations (10, 5, 2.5, 1.25, 0.63, 0.31 g rubber/L dilution water). Duplicated dilution series (series a and b) were prepared from all the samples, and duplicated dilution series of the reference sample accompanied each test run. One sample (sample 4) was also tested in a second

Table 1 Composition of the different rubber samples

Test sample (sample no. in this study)	Components and additive(s)	Concentration of the components (%) and comments on preparation
Reference sample	Rubber polymer:	
(1)	SBR 1500; 23.5% styrene and 76.5% butadiene	64
	Reinforcing agent:	
	Carbon black; N-234	32
	Vulcanization activators:	
	Zinc oxide	1.28
	Stearic acid	0.96
	Vulcanization agent:	
	Sulphur	1.12
	Accelerator:	
	CBS; N-cyclohexyl-2-benzothiazolesulfenamide	0.64
Process oils		
(2)	**TDAE1**; treated distillate aromatic extract 1	16% of the different process oils were added to the reference sample to prepare samples 2 to 6, respectively.
(3)	**TDAE2**; treated distillate aromatic extract 2	
(4)	**HA**; high aromatic oil	
(5)	**MES**; mild extraction solvate	
(6)	**NPO**; naphtenic process oil	
Antidegradents		
(7)	**TMQ**; 2,2,4- trimethyl-1,2-dihydroquinoline	1.9% of the different antioxidants/ antiozonants were added to the reference sample to prepare samples 7 to 12, respectively.
(8)	**6PPD**; N-(1,3-dimethylbutyl)-Ń-phenyl-p-phenylenediamine	
(9)	**IPPD**; N-isopropyl-Ń-phenyl-p-phenylenediamine	
(10)	**6PPD+TMQ**	
(11)	**Wingstay L**; Phenol, 4-methyl-reaction products with dicyclopentadiene and isobutylene	
(12)	**DTPD**; Chemical composition mixture of diaryl-p-phenylenediamine	
Accelerators		
(13)	**MBS**; 2-(4-morpholinothio)benzothiazole	CBS in the reference sample was replaced by the different benzothizole accelerators (0.64%) to prepare samples 13 to 15, respectively.
(14)	**TBBS**; N-tert-butyl-2-benzothiazolesulfenamide	
(15)	**MBTS**; 2,2́-benzothiazyldisulfide	
(16)	**DPG**; 1,3-diphenylguanidine	0.19% DPG was added to the reference sample.
(17)	**TMTD**; thiram	0.064% TMTD was added to the reference sample.
(18)	**DTDM**; 4,4́-dithiodimorpholine	Some of the sulphur in the reference sample was replaced by 0.64% DTDM.
Fillers		
(19)	**Silica+silane**	16% silica and 1.28% silane replaced 16% of the N-234 in the reference sample.
(20)	**$CaCO_3$**	32% $CaCO_3$ and silica replaced the N-234 in the reference sample to prepare samples 20 and 21, respectively.
(21)	**Silica**	
(22)	**Silica+silane**	N-234 in the reference sample was replaced by 32% silica and 2.56% silane.

test run with four replicated dilution series to check the method's replicability. The dishes were left at room temperature for 72 h to allow the rubber to equilibrate with the water before test organisms were added. Acute toxicity tests were performed with *Daphnia magna* as test organisms, according to ISO (1996). Fifteen to 20 neonates (0–24 h) were added to each dish containing leachate water and rubber pieces. A positive control with a reference toxicant ($K_2Cr_2O_7$) and a negative control, containing only dilution water, accompanied each test. The temperature during testing was 20 ± 2°C and the photoperiod used was 16 h light:8 h darkness. Immobility was recorded after 24 and 48 h. Leachates with the surviving test organisms were then irradiated with ultraviolet (UV) light (295–365 nm with peak emission at 340 nm) for 2 h as described by Wernersson and Dave (1997), and immobility was again recorded after another hour.

EC50 values were calculated with a software program that uses both the moving average and the probit methods (Peltier and Weber, 1985); the presented values were calculated with the method giving the narrowest confidence interval. Percentages of immobility were transformed using the arcsine of the square root before analysis of variance (ANOVA) was applied using a software program (SPSS Base, ver. 11.5.1, SPSS Inc., Chicago, IL, USA), to test for differences in toxicity among samples. Significant differences were assessed by the Student–Newman–Keuls post hoc test, and significance was defined as $P \leq 0.05$.

Results and Discussion

Immobility in dilution water controls was less than 10% in all cases. The 24-h EC50s for the reference toxicant ($K_2Cr_2O_7$) was 0.7–0.9 mg/L in the six test runs, which is within the limits prescribed by ISO (1996). For several leachates there were poor concentration–response relationships with very flat curves, which made it difficult to calculate EC50 values. This may have been caused by different leaching potentials at different loading rates. Figure 1 gives an overview of the toxicity of all the samples, shown as the total percentage of immobilized daphnids in all concentrations. A compilation of all the results is given in Table 2, where the toxicities of the duplicated dilution series (a and b) of all the samples are presented both as the total percentage of immobilized daphnids, and as EC50 values (gram rubber per litre dilution water) when they could be calculated. Table 2 also shows the effect of UV irradiation, expressed as the percentage of irradiated daphnids that were immobilized. The 24-h EC50 values varied more than 20-fold, ranging from >10 g/L for the least toxic samples to 0.5 g/L (average of duplicates) for the most toxic sample (sample 9). After 48 h of exposure and after UV exposure, the variability was more than 22-fold, with the least toxic sample (sample 18) having an EC50 value of 7.0 g/L, and the most toxic sample (sample 9) a value of <0.31 g/L. A one-way ANOVA was conducted to explore the impact of rubber sample ($n = 22$) on toxicity after 24 and 48 h exposures, and after UV exposure. There was a significant effect for the kind of rubber (test sample) at all exposure times. The post hoc comparisons indicated that sample 9 was more toxic than all other samples after 24 and 48 h of exposure, and that samples 9 and 12 were more toxic than all other samples after UV exposure. Consequently, the most toxic samples contained antidegradents of para-phenylenediamine type, sample 9 contained N-isopropyl-N-phenyl-p-phenylenediamine (IPPD) and sample 12 contained diaryl-p-phenylenediamine (DTPD). Antidegradents function through sequential migration to the surface, which is initiated by a disruption in equilibrium within the rubber mix caused by the use of the chemical at the rubber surface (Ignatz-Hoover et al., 2003); this migration behavior might also explain the high toxicity for some of the samples within this group. The three samples containing benzothiazole accelerators (samples 13, 14, and 15) were among the five most toxic samples at all exposure times. Benzothiazoles have been used as tracers for tire contamination (Spies et al., 1987).

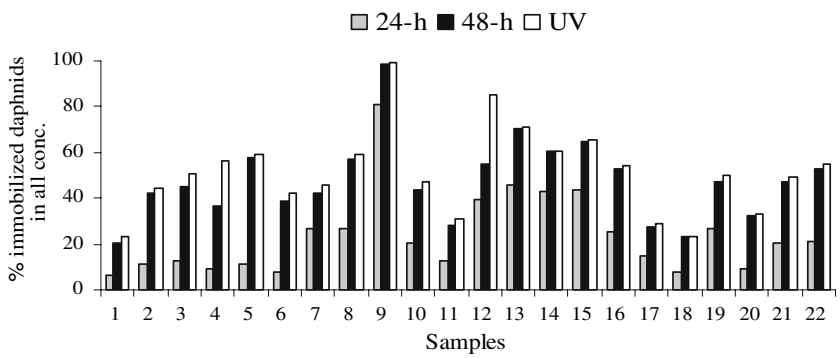

Fig. 1 Total percentage of immobilized daphnids in all six concentrations of leachates of the reference sample 1 (mean of 10 replicates) and of samples 2–22 (mean of duplicates)

Table 2 Toxicity to *Daphnia magna* of the reference sample (sample 1) and of rubber samples 2–22, expressed as the total percentage of immobilized daphnids in all concentrations and as EC50 value (gram rubber per litre dilution water) when it could be calculated. Confidence intervals (95%) are shown in parentheses. Samples 2–22 were tested in duplicated dilution series, a and b. The last column shows the percentage of UV-irradiated daphnids that were immobilized. Samples which were significantly more toxic than the reference are shown in bold

Test sample[a]	24 h		48 h		+ UV
	Imm. (%)	EC50	Imm. (%)	EC50	% of irradiated daphnids immobilized
1	6.3		20.6		4
	±7.3[b]		±11.8[b]		±2.7[b]
2a	9	>10	47		4
2b	13	>10	38		**7**
3a	16	>10	37		11
3b	9	>10	53		10
4a	8	>10	37		**30**
4b	11	>10	36		**33**
	0[c]	>10	14[c]		**42**[c]
	3[c]	>10	18[c]		**43**[c]
	2[c]	>10	17[c]		**33**[c]
	0[c]	>10	18[c]		**44**[c]
5a	12	>10	**49**		2
5b	11	>10	**67**		3
6a	8	>10	38	2.9 (2.3–3.7)	4
6b	8	>10	32	2.6 (2.3–3.5)	7
7a	23	5.9 (4.3–9.6)	43		6
7b	30		43		3
8a	28		57	1.4 (1.0–1.8)	2
8b	26		57	1.1 (0.9–1.5)	8
9a	**78**	0.6 (0.4–0.7)	**99**	<0.31	–
9b	**84**	0.4 (0.3–0.5)	**98**	<0.31	–
10a	19	>10	41		10
10b	23	6.0 (4.5–9.0)	46	2.1 (1.6–2.7)	3
11a	17	>10	28		7
11b	8	8.0 (6.3–12)	28		2
12a	**42**	2.5 (1.8–3.3)	**56**	1.4 (1.2–1.8)	**66**
12b	**37**	3.3 (2.7–4.3)	**54**	1.5 (1.2–2.0)	**62**
13a	**51**		**73**		4
13b	**40**		**68**		0
14a	**46**		**60**		0
14b	**40**		**61**		0
15a	**42**	2.7 (1.9–4.2)	**57**	1.2 (0.8–1.8)	4
15b	**46**		**73**	0.5 (0.3–0.8)	0
16a	28	5.2 (3.6–8.8)	52	1.7 (1.3–2.1)	2
16b	23		53	1.6 (1.1–2.1)	5
17a	11	10.6 (7.8–22.7)	23	5.9 (4.6–8.0)	3
17b	19	7.1 (6.0–8.4)	32		1
18a	8	>10	21	7.8 (5.2-10.0)	0
18b	7	>10	26	7.1	0
19a	26	5.4 (4.0–7.5)	48		5
19b	27	5.0 (3.6–8.1)	47		5
20a	8	>10	25	6.8 (4.8–7.3)	1
20b	10	9.6	40	3.2 (2.1–5.6)	3

Table 2 continued

Test sample[a]	24 h		48 h		+ UV
	Imm. (%)	EC50	Imm. (%)	EC50	% of irradiated daphnids immobilized
21a	23	>10	46	2.2 (1.6–3.1)	5
21b	18	7.8 (6.1–12.3)	48		8
22a	15	>10	43		4
22b	27	>10	62		4

[a] Samples as described in Table 1

[b] Mean ± SD of the results for the reference sample tested in different test runs (total $n = 10$)

[c] Tested in the second test run of sample 4

Samples that were significantly more toxic than the reference sample are shown in bold in Table 2. Altogether, six of the 21 tested samples were more toxic than the reference sample after 48 h exposure, indicating that several additives used in tread rubber can migrate from the rubber to water after relatively short leaching times (72 hours), in forms that are bioavailable. However, the reference sample was also slightly toxic, with 21% immobilization in all concentrations (mean of ten replicates) after 48-h exposure. The reference sample contained zinc oxide (at a concentration of 1.28%, which is similar to the zinc content in tires), and previous studies have shown water extracts of shredded rubber/tire wear material to contain high concentrations of zinc (Anthony et al., 1995; Gualtieri, 2005; Wik and Dave, 2006). Zinc has also been shown to be the main reason for toxicity to *Ceriodaphnia dubia* (Nelson et al., 1994). Therefore, it is probable that zinc caused the observed toxicity of the reference sample.

The migration behaviors of different additives are dependent on the nature of the additive and its solubility and reactivity with other components, i.e., the rubber composition (Ignatz-Hooever et al., 2003). This means that it cannot be concluded that only the particular additive caused the observed toxicity. Rather the observed toxicity is the combined result of the background toxicity observed in the reference sample and the leachability and inherent toxicity of each additive. It is also worth emphasizing that differences in dilution water quality characteristics could lead to different leaching potentials of compounds from the rubber matrix as well as alterations in toxicity due to, for example, interactions between toxicants and components in the dilution water. A leaching test with rubber granulates did, for example, indicate a lower leachability of polycyclic aromatic hydrocarbons (PAHs) (sum-PAH) and a higher leachability of zinc in more-alkaline water compared to distilled water (Westerberg and Mácsik, 2001). On the other hand, a decrease in zinc toxicity could be expected in harder and more-alkaline water due to speciation and complexation of the zinc ion (Heijerick et al., 2002).

An ANOVA was conducted to evaluate the effect of photoactivation of different samples (the percentage of irradiated daphnids that were immobilized). Daphnids exposed to samples 4 and 12 were significantly more affected by UV irradiation than daphnids exposed to the other samples. Sample 4 contained a high level of aromatic oil and the increased toxicity due to UV irradiation was probably caused by polycyclic aromatic hydrocarbons (PAHs), which are present at high concentrations in this type of oil (Cedheim et al., 2001). Several PAH species have been shown to be phototoxic (Wernersson and Dave, 1997). However, the results of this study also imply that the phototoxicity of tire leachates may also be due to antidegradents such as DTPD.

The EC50 values for the most toxic samples in this study are similar to the values that we have found for the most toxic tires in previous studies (Wik and Dave, 2005; Wik and Dave, 2006). The same additives included in the most toxic rubber samples in this study are therefore likely to be the main toxicants in tires as well. Gualtieri and coworkers (2005) reported 48-h EC50 values for leachates of tire wear that are about 100-fold higher compared to the most toxic tires/rubber samples in our tests. In their study, however, only one tire was tested, which could explain some of the variation since rubber formulation, as shown in this study, produces a variation in toxicity of about 20-fold. The rest of the variation in toxicity between different studies on tire leachates is probably due to different leaching procedures (loading rates, dilution water and leaching times). To summarize, the findings from this study have shown that the choice of chemical additives in rubber greatly affects the toxicity. This should be taken into consideration in the further development of rubber for tires to reduce their environmental impact.

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