Comparison of extraction materials and genotoxicity tests for the analysis of UV/H_2O_2-treated water

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KWR Watercycle Research Institute

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Comparison of extraction materials and genotoxicity tests for the analysis of UV/H_2O_2-treated water - draft

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Summary

Recent reports on the analysis of genotoxicity in UV/H_2O_2-treated water indicate that the choice of extraction method and of the genotoxicity test may have a crucial impact on the outcome of the analysis. KWR reported to find formation of genotoxicity by UV/H_2O_2-treatment by medium pressure (MP) lamps at three different locations when extracting the water with Oasis® HLB and performing an Ames fluctuation test, but not when performing a comet assay with human liver cells. HWL reported not to find formation of genotoxicity when extracting MP UV/H_2O_2-treated water with XAD-4 and performing an Ames plate test, but to see an increase in genotoxicity when analyzing exposed fish gill cells with the comet assay. Greater Cincinnatti Water Works (GCWW) also reported to see formation of genotoxicity by MP UV/H_2O_2-treatment with the comet assay in Chinese hamster ovary cells (CHO), when extracting the water with XAD-8 and XAD-2. The influence of the applied extraction method and genotoxicity test on the detection of genotoxic by-products of UV-oxidation of water was therefore investigated. Specific aims were to:

1. Compare Oasis® HLB with XAD-4 in the resulting response of MP UV/H_2O_2-treated water on the Ames fluctuation test, the Ames plate test, and the comet assay with human liver cells
2. Compare the response of the Ames fluctuation test and the Ames plate test using the same extracts of MP UV/H_2O_2-treated water
3. Compare XAD-4 and XAD-2+ XAD-8 in the resulting response of MP UV/H_2O_2-treated water on the comet assay with human liver cells
4. Compare coconut charcoal and Oasis® HLB on the resulting response of MP UV/H_2O_2-treated water on the Ames fluctuation test including TA100, as nitrosamines are better extracted by coconut charcoal.

With respect to these aims, this study has found that:
1. Oasis® HLB extracts the genotoxic compounds in MP UV/H_2O_2- treated water best, but XAD extracts these compounds quite well, too. The difference in extraction method can therefore not explain the different outcomes seen by KWR and HWL.
2. The Ames plate test and the Ames fluctuation test both detected a dose-related response in extracts of the MP UV/H_2O_2- treated water, which was above the threshold of significance at the employed reference doses in both test versions. Based on the TTC, in both test versions health risks cannot be excluded for water producing such a response. The two test versions therefore appear to agree well in the analysis of this MP UV/H_2O_2- treated water, which does not provide an explanation for the different results found by KWR and HWL.
3. No genotoxic responses were detected in the XAD-4 and XAD-2 + XAD-8 extracts of the MP UV/H_2O_2- treated water in the comet assay. This again provides no explanation for the differences found by KWR and GCWW.
4. The coconut charcoal extract did not cause any genotoxic response, in contrast to the Oasis® HLB extract. This indicates the observed genotoxicity in Oasis® HLB extracts is not caused by hydrophilic nitrosamines, as coconut charcoal extracts these well. These results might also be an indication that the genotoxic substances in MP UV/H_2O_2- treated water are not removed by adsorption in GAC, but by another process, e.g. microbial degradation.

Because of the good performance compared to the other methods, and the practical advantages, the Ames fluctuation test with Oasis® HLB extraction is preferred for the genotoxicity analysis of UV/H_2O_2-treated water.

None of the different results found by the earlier reports could be explained by this comparison study. This comparison study was performed in one laboratory. Possibly, small differences in procedure steps as performed by the different laboratories, employed by the different earlier reports, are responsible for the observed differences. It happens regularly that different laboratories find different results, without a clear cause. To verify if the differences come from the employment of different laboratories, a small round robin study could be undertaken to have the different laboratories extract duplicates of the same water, and exchange the duplicate extracts for genotoxicity analysis to make a cross-comparison.

Additionally, an exchange of technicians could be made, to enable discovery of small differences in methods.

Contents

1 Introduction

Previously, KWR has reported on the detection of increased genotoxic activity in water after medium pressure (MP) UV-oxidation treatment, as measured after water extraction with Oasis™ HLB solid phase extraction (SPE) columns and analysis with the Ames fluctuation mutagenicity test (Heringa *et al.*, 2011; BTO 2011.046). This was observed in three different water sources, in both pilot plants and a full scale plant. No genotoxic activity was measured in the same samples in the comet assay using human liver cells. The comet assay is a complementary genotoxicity test to the Ames fluctuation test, detecting different DNA damage and thus different genotoxic compounds. In contrast, Penders *et al.* (IUVA 2009) of HWL found no genotoxic activity in MP UV/H_2O_2-treated water from one of the same sources after water extraction with XAD-4 and analysis with the Ames plate test. They did find an increased genotoxic response in the gill cells of fish exposed to this treated water, analyzed with the comet assay. Kashinkunti *et al*. (WQTC 2009) of Greater Cincinnatti Water Works (GCWW)also reported an increase in genotoxic response after MP UV-treatment with an oxidation-level dose, in the comet assay with a Chinese hamster ovary (CHO) cell line. They used one of the same sources as in the Heringa *et al.* study, but Kashinkunti *et al*. extracted the water with XAD-2 and XAD-8.
These reports indicate that the choice of extraction method and of the genotoxicity test may have a crucial impact on the outcome of the analysis. The influence of the applied extraction method and genotoxicity test on the detection of genotoxic by-products of UV-oxidation of water was therefore investigated. Specific aims were to:

1. Compare Oasis® HLB with XAD-4 in the resulting response of MP UV/H_2O_2-treated water on the Ames fluctuation test, the Ames plate test, and the comet assay with human liver cells
2. Compare the response of the Ames fluctuation test and the Ames plate test using the same extracts of MP UV/H_2O_2-treated water
3. Compare XAD-4 and XAD-2+ XAD-8 in the resulting response of MP UV/H_2O_2-treated water on the comet assay with human liver cells

Additionally, the effect of extraction material and organic solvent on the possible detection of nitrosamines was investigated. It was postulated by Helma *et al.* (1994) that nitro or nitroso compounds could be formed during UV-irradiation if water in presence of nitrite and nitrate. One well-known type of nitroso compounds, that are known to be genotoxic, are the nitrosamines. NDMA (N-nitroso-dimethylamine) is a nitrosamine already well-investigated in drinking water as it is known to be formed from chlorination and chloramination of water (Richardson and Ternes, 2011). NDMA and other hydrophilic nitrosamines, however, have been found not be extracted well by Oasis® HLB (Krauss and Hollender, 2008), and will presumably therefore not have been present in the extracts of Heringa *et al*. (2011). Nitrosamines are better extracted with coconut charcoal (e.g. Munch and Bassett, 2006). Furthermore, NDMA is not responsive in the Ames test in TA98 (+ and – S9) or in TA100-S9, only in TA100+S9 (NTP database). TA100 was not included in the test of Heringa *et al*. (2011).

Therefore, an additional aim was to:

4. Compare coconut charcoal and Oasis® HLB on the resulting response of MP UV/H_2O_2-treated water on the Ames fluctuation test including TA100.

2 Methods

2.1 Preparative experiment 1: genotoxicity check of water and effect of quenching substance

The best available MP UV/H_2O_2-treated water for the comparison experiments was the water from a pilot installation of Dunea at Bergambacht. This pilot installation was fed with pre-treated (coagulation, microstraining and rapid sand filtration) Meuse river water from the full scale treatment plant, as used in Heringa *et al*. (2011). The pilot effluent water had not been tested for genotoxicity yet, while it was crucial for the comparison experiment that the water would contain a similar genotoxic activity as seen in Heringa *et al*. (2011). Therefore, the water before and after MP UV/H_2O_2-treatment was first tested for genotoxicity with the same methods as in Heringa *et al*. (2011). Simultaneously, some other effects were studied:

- the effect of excluding H_2O_2-dosing, as excluding H_2O_2-dosing had previously been shown to increase the formation of genotoxic activity by UV-treatment, but this had not been reproduced yet (Heringa *et al*., 2011).
- the effect of low pressure (LP) lamps on the formation of genotoxic activity
- the effect of adding sodium sulphite (Heringa *et al*., 2011) or catalase (Penders *et al*., IUVA 2009) or nothing, to quench residual H_2O_2, on the formation of genotoxic activity.
- the effect of GAC on the removal of any formed genotoxic activity, as already seen in Heringa *et al*. (2011).

On 12 November 2009, water samples of one litre were collected in extensively cleaned glass bottles at the pilot installation of Dunea at Bergambacht, at the points given in Table 1, after which quenching substance was added as also indicated in Table 1. The catalase from bovine liver was purchased from Sigma (C100-500MG) and came as a solution of 31,25 mL, with 16 mg protein/mL (42600 U/mg protein). This solution was diluted 10-fold with milliQ water. As Penders *et al*. (IUVA 2009), 8000 U was dosed per litre of sampled water, i.e. 117 µL of catalase dilution.

Table 1. Samples collected for preparative experiment 1 and additions of quenching substance

Sample	Addition of quenching substance (per L)
Post SF	300 mg sodium sulphite
Post MP UV	Nothing
	300 mg sodium sulphite
	117 µL catalase-dilution
Post MP UV/H_2O_2	Nothing
	300 mgL sodium sulphite
	117 µL catalase-dilution
Post LP UV	300 mg sodium sulphite
Post LP UV/H_2O_2	300 mg sodium sulphite
Post MP UV/ H2O2-GAC	300 mg sodium sulphite
Evian mineral water (Blanc)	Nothing
	300 mg sodium sulphite
	10 mg H_2O_2

The pilot-scale installation of Dunea was built with a design flow of 5 m^3/h per reactor. The LP reactor (LBX 10) was obtained from ITT Wedeco (Herford, Germany) and was equipped with four 330 W lamps with an automatic wiping system, thus rendering a total installed power of 1.32 kW. The MP reactor (B 2020) was obtained from Berson UV Technology (Nuenen, The Netherlands) and was equipped with two 2200 W lamps with an automatic wiping system, thus rendering a total installed power of 4.4 kW. Both the MP UV-reactor as the LP UV-reactor were set at 100% power, H_2O_2 was dosed at 10 mg/L H_2O_2.

These settings had previously been found to give an average of 78% and 72% conversion of atrazine, respectively (Lekkerkerker *et al*., accepted), and was therefore deemed the best setting to mimic a full-scale application.
The water samples were transported to KWR on ice, stored at 4°C and extracted within 24 hours as in Heringa *et al*. (2011). One litre of each sample was brought to pH 2.3 and extracted with 200 mg Oasis™ HLB glass columns with a sand filter on top. The columns were eluted with 3 times 2.5 mL acetonitrile: methanol 80:20. These extracts were evaporated and redissolved in 100 µL DMSO and then stored at -18°C until analysis with the Ames fluctuation test. A full description of the extraction procedure can be found in Appendix I. The Ames fluctuation test was performed with TA98 and TAMix, both + and – S9, as described in Heringa *et al*. (2011) and in Appendix II.

2.2 Preparative experiment 2: increasing extraction volume

In the comparison experiment, 50 L of MP UV/H_2O_2-treated water needed to be extracted with Oasis™ HLB to produce enough extract for the Ames plate test, while the 200 mg-columns used until then could only take 1 L of water each, and the manual setup maximally 16 columns a day. The resulting through-put of 16 L a day, thus 4 days for at least 50 L, would not be very efficient. Therefore, a preparative experiment was performed to study the extraction performance (with respect to the genotoxic substances in MP UV/H_2O_2-treated water) of 500 mg Oasis™ HLB plastic columns. These columns can take up to 2 L of water each, but are not available in the more inert material of glass, only in plastic. Plastic can absorb substances. Additionally, the extractions were performed with a Gilson (Middleton, WI, U.S.A.) Trilution LH automatic extraction device, which can handle 32 columns a day. With 2 L per column and 32 columns a day, 64 L a day could be extracted. The 200 mg Oasis™ HLB glass columns, however, do not fit on this automatic extraction device. Therefore, these were applied using the manual method with a manifold.
As a different research project had found that Oasis™ MCX (i.e. Oasis™ HLB mixed with a cation exchange material) performed slightly better in the average extraction of a wide range of compounds (report in preparation), 500 mg Oasis™ MCX plastic columns were also included in the experiment. The aim here was to see whether the specific genotoxic substances in MP UV/H_2O_2-treated water are also better extracted with Oasis™ MCX than with Oasis™ HLB.
Water was sampled after MP UV-treatment without H_2O_2 of sand-filtered water on 1 February 2010 at the pilot installation of Dunea at Bergambacht (conditions as described in 2.1). This water was extracted in three different ways, as shown in Table 2. A power-calculation indicated that, estimating a coefficient of variation of 10%, 23 replicates would be necessary to detect a difference of 10% between the Ames fluctuation test responses of the extracts. This was not feasible for a comparison of extraction methods on the same day (to ensure the same water quality). Therefore, a compromise was found with 5 replicates, which would detect a difference of 25% in the Ames fluctuation test responses between the extracts. Thus, 5 replicate extractions were performed with each type of column to ultimately make 5 replicate extracts of 100 µL DMSO from 1 L of water (10,000-fold concentrated) for the Ames fluctuation test. Additionally, 2 replicate extracts of 100 µL DMSO from 2 L of water (20,000-fold concentrated) were prepared for the comet assay. As no response had been detected in extracts from MP UV/H_2O_2-treated water in the comet assay before, it was not deemed necessary to test more replicates in this assay. Both the 7,5 mL-extracts from the manual method and the 10 mL-extracts from the automatic method were evaporated and redissolved in DMSO to 20,000-fold concentrated extracts as described in Appendix I. The 100 µL extracts of 20,000-fold concentration from the 500 mg columns were then split into 50 µL for the Ames fluctuation test, which was diluted with DMSO to 100 µL, and into 50 µL for the comet assay, which was pooled with one other replicate to make 100 µL.
The Ames fluctuation test was performed with TA98 and TAMix, both + and – S9, as described in Heringa *et al*. (2011) and in Appendix II. The comet assay was performed with HepG2 human liver cells at 3h and 24 h exposure, without S9. The procedure details of the comet assay can be found in Appendix III. As there was no statistical test known to compare two different samples with binomially distributed data, but it was known the binomial data approach normally distributed data at higher counts, a two-tailed type 2 t-test was performed to compare the responses from the different extracts.

Table 2. Extraction details for experiment 2.

Sample	SPE column	Extraction method	Elution[1]	n
Post MP UV	200 mg Oasis™ HLB in glass	manual, 1 L/column	3x 2,5 mL ACN: methanol 80:20	9
	500 mg Oasis™ HLB in plastic	automatic, 2 L/column	10 mL ACN: methanol 80:20 at 1 mL/min	5
	500 mg Oasis™ MCX in plastic	automatic, 2 L/column	3 mL ACN at 1 mL/min, then 7 mL ACN with 5% NH_4OH at 1 mL/min	5
Procedure control (no water)	200 mg Oasis™ HLB in glass	manual, 1 L/column	3x 2,5 mL ACN: methanol 80:20	3
	500 mg Oasis™ HLB in plastic	automatic, 2 L/column	10 mL ACN: methanol 80:20 at 1 mL/min	2
	500 mg Oasis™ MCX in plastic	automatic, 2 L/column	3 mL ACN at 1 mL/min, then 7 mL ACN with 5% NH_4OH at 1 mL/min	2

[1] ACN = acetonitrile

2.3 Comparison experiment-part 1

In the first part of the comparison experiment, UV/H_2O_2-treated water was extracted with XAD-4, Oasis® HLB and coconut charcoal and tested with the Ames plate test and Ames fluctuation test. The extracts prepared for the comet assay were stored until the second part of the comparison experiment.

2.3.1 Sampling

Water was sampled after MP UV/H_2O_2-treatment of sand-filtered water on 21 September 2010 at the pilot installation of Dunea at Bergambacht. The UV-reactor was set at 100% power, H_2O_2 was dosed at 10 mg/L H_2O_2. Six extensively cleaned 35-L stainless steel barrels were filled, after which 10,5 g Na_2SO_3 was added to each barrel to quench the residual H_2O_2. The barrels were transported to KWR and kept at room temperature, to ensure these large volumes of water had reached the required room temperature at the time of extraction the following day.

2.3.2 Sample extraction with XAD-4 and concentration

Two 50-litre samples of the water were extracted with pre-purified Amberlite XAD-4 (Rohm & Haas) at ambient pH and subsequently at pH 2 according to the protocol described in reports SWI 88.111 and SWI 89.122. In brief, a 50-L water sample was passed through two serial columns with 50 mL of purified XAD-4 overnight at 50 mL/min, with an acidification step to pH 2 after the first column. One procedure control, consisting of an extraction without water, was included. Remaining water was pressed out of the column with nitrogen, after which the columns were washed with 500 mL milli-Q water (acidified to pH 2 with HCl for the pH 2 column) to remove the salts from the dried columns. After another drying step, the extracted compounds were eluted from each of the columns with 250 ml ethanol and subsequently 250 mL ethanol: cyclohexane 30:70 (both J.T. Baker, >99.9% purity) in around 2 hours. The eluate was directly filtered using a 0,45 µm Teflon membrane filter (Sartorius) to remove microorganisms that could affect the Ames test. Then, 570 mL of cyclohexane was added to obtain an azeotropic mixture from which the water is removed first during the concentration step. The diluted eluate was concentrated by an overnight evaporation to 250 mL at solvent boiling point (56-78 °C), then further down to 5 mL at ethanol boiling point (78 °C). Finally, the extract was concentrated to 2 mL under a nitrogen stream at around 60 °C, reaching a concentration factor of 25,000. The extracts of the two water samples were pooled to yield 4-mL extracts of each pH. Then, each pooled extract was split into 2 mL for the Ames plate test, 80 µL for the Ames fluctuation test (diluted to 200 µL to yield a 10,000-fold concentrated extract), 80 µL for the comet assay (diluted to 100 µL to yield a 20,000-fold concentrated extract), and a remainder.

The procedure control (i.e. extraction without water) followed the same steps, but only 1 mL of "extract" was prepared for the Ames plate test, as only the highest dose would be tested. All extracts were stored at -18°C until analysis. The extraction details are also summarized in Table 3.

Table 3. Overview of the extraction and concentration procedures and the performed genotoxicity tests with these extracts.

Sample	Extracted sample volume (L)	Extraction method	Concentration specifics	Final extracts	Applied genotoxicity tests
Post MP UV/H_2O_2 (210 L)	50	SPE with XAD 4 at pH neutral and pH 2 (separate extracts)	Extracts of each pH pooled, evaporated to 2 mL (25.000x)	2 mL ethanol (25.000 x)	Ames plate test (with different extract doses)
	50	SPE with XAD 4 at pH neutral and pH 2 (separate extracts)		200 uL ethanol (10.000x)	Ames fluctuation test (with different extract doses)
				100 uL ethanol (20.000x)	Comet assay (1 dose)
	50	SPE with Oasis® HLB	Extracts pooled, evaporated, redissolved in DMSO to 25.000x	2 mL DMSO (25.000x)	Ames plate test (with different extract doses)
	2	SPE with Oasis® HLB		200 uL DMSO (10.000x)	Ames fluctuation test (with different extract doses)
	2	SPE with Oasis® HLB		100 uL DMSO (20.000x)	Comet assay (1 dose)
	2	SPE with Oasis® HLB	Evaporated and redissolved in ethanol	200 uL ethanol (10.000x)	Ames fluctuation test (with different extract doses)
	2	SPE with coconut charcoal	Evaporated and redissolved in ethanol	200 uL ethanol (10.000x)	Ames fluctuation test (with different extract doses)
Procedure control (no water)	"50 L"	SPE with XAD 4 at pH neutral and pH 2 (separate extracts)	Evaporated down to 2 mL ethanol (25.000 x)	1 mL ethanol (25.000x)	Ames plate test (with 1 dose)
				200 uL van 10.000x	Ames fluctuation test (with 1 dose)
				100 uL van 20.000x	Comet assay (1 dose))
	"26 L"	SPE with Oasis® HLB	Extracts pooled, evaporated, redissolved in DMSO to 2.16 mL (25.000x)	1 mL DMSO (25.000x)	Ames plate test (with 1 dose)
	"2 L"	SPE with Oasis® HLB		200 uL DMSO (10.000x)	Ames fluctuation test (with 1 dose)
	"2 L"	SPE with Oasis® HLB		100 uL DMSO (20.000x)	Comet assay (1 dose)
	"2 L"	SPE with Oasis® HLB	Evaporated and redissolved in ethanol	200 uL ethanol (10.000x)	Ames fluctuation test (with 1 dose)
	"1 L"	SPE with coconut charcoal	Evaporated and redissolved in ethanol	100 uL ethanol (10.000x)	Ames fluctuation test (with 1 dose)

2.3.3 Sample extraction with Oasis® HLB and coconut charcoal and concentration

The detailed procedure of the extraction with Oasis® HLB can be found in Appendix I. In brief, 28 replicates of two litres of sample were extracted by solid phase extraction (SPE) with 500-mg Oasis® HLB cartridges (Waters Corporation, Milford, USA) at pH 2.3 on a Gilson (Middleton, WI, U.S.A.) Trilution LH automatic extraction device. Another 16 columns were used as procedure controls, consisting of an extraction without water.
Elution was performed with addition of 10 mL of 20% methanol in acetonitrile at 1 mL/min. All but one of the 10-mL eluates were pooled, evaporated to near dryness and then dissolved in DMSO to yield 2.16 mL of a 25,000-fold concentrated extract. A fraction of 2 mL of this pooled extract was used for the Ames

plate test, having the same water concentration as the XAD-extracts. Another fraction (80 µL) was diluted with DMSO to yield 200 µL of a 10,000-fold concentrated extract for the Ames fluctuation test. A third fraction of 80 µL was diluted with DMSO to yield 100 µL of a 20,000-fold concentrated extract for the comet assay.
The one remaining 10-mL eluate was evaporated to dryness and redissolved in 200 µL ethanol (10,000-fold concentrated) for an Ames fluctuation test, to compare with the coconut charcoal extract. Procedure controls followed the same steps, but only 1 mL of 25,000-fold "extract" was prepared for the classic Ames test, as only the highest dose would be tested. All extracts were stored at -18°C until analysis.

Two litres of the sampled water were extracted with activated coconut charcoal (Restek, Bellefonte, PA, U.S.A.) according to protocol LOA-535-nitrosamines. The final dichloromethane eluate was evaporated and redissolved in 200 µL ethanol, which was stored at -18°C until analysis.

An overview of all extraction procedures is given in Table 3.

2.3.4 Ames plate test

The test strains (TA98 and TA100) were purchased from Xenometrix (Allschwil, Switzerland). For the Ames plate test, protocol LMB-015 was followed, which is in accordance with OECD guideline 471, except that only strains TA98 and TA100 were used. Some small deviations to the protocol were made, specific for this study, which are underlined in the description below.
In brief, overnight cultures of the obtained frozen bacteria were prepared in Nutrient Broth No. 2. Then, 0,1 mL of the overnight culture, 0,5 mL of S9-mixture or of a buffer-mixture, and 10, 20, 40, 60 or 80 µL of water extract was mixed with 3,0 mL of warm topagar, which was immediately swirled on a 20-mL layer of solidified bottomagar in a petri dish. The picrolonic acid as a positive control for TA98 –S9, according to the protocol, was replaced by 2 µg/plate 4-nitroquinoline, as picrolinic acid was not available in the desired purity. The water extracts were tested in duplicate in both strains (TA98 and TA100), both with and without the liver enzyme extract S9[1]. Procedure controls were tested only at the 80 µL dose
The petri dishes with solidified topagar were then incubated for 65 hours at 37 °C. After 65 hours, the number of colonies were counted on each petri dish as the measure of genotoxicity. Cytotoxicity was assessed by microscopically judging the background microbial growth, to check for possible artifacts due to effects on cell survival and growth. A sample was considered genotoxic if the number of induced revertants (i.e. the number of revertants of the sample subtracted with the number of revertants of the negative control) was at least twice the number of revertants of the negative control (i.e. the number of "spontaneous revertants"). A dose-response should also be shown to be certain the observed effect is indeed a genotoxicity effect. Cytotoxicity was checked by inspection of the presence of the normal background "lawn" of bacteria on the plate.

2.3.5 Ames fluctuation test

The Ames fluctuation test strains (TA98 and TA100) and media were purchased from Xenometrix. The test procedure provided by Xenometrix, also described by Fluckiger-Isler *et al*. (2004), was followed, with minor modifications as described in Appendix II. In brief, 2,3,4,5, or 6 µL of a water extract (and additional solvent to give a total of 6 µL) was added to 294 µL of a mixture of bacterial overnight culture, S9-mixture if appropriate, and medium, so the bacteria were finally exposed to a 200-fold concentration of the water samples in culture medium and 2% solvent. Water extracts were tested in duplicate, as well as a triplicate negative control (solvent only), a triplicate positive control for genotoxicity, and a triplicate positive control for cytotoxicity. Procedure controls were tested in triplicate, at the highest dose of 6 µL. All extracts were tested in TA98 and TA100, both with and without the liver enzyme extract S9[1].
After 90 minutes incubation, 2.7 mL of indicator medium, containing bromocresol purple as indicator, was added and the mixture was divided over 48 wells of a 384-well plate (50 µL per well). These plates were incubated for 48 hours at 37 °C. Finally, the number of yellow wells per 48 wells of one sample were counted manually as a measure of genotoxicity. A custom cytotoxicity test was performed with

[1] The Ames test is performed both with and without S9 liver enzyme extract, in order to detect both direct genotoxic compounds, and indirect genotoxic compounds that need to be converted to a genotoxic metabolite by liver enzymes first.

subsamples of the exposure cultures in medium with histidine, to check for possible artefacts due to effects on cell survival and growth.
Ames test responses follow a binomial distribution (Piegorsch *et al*., 2000), therefore a sample was considered genotoxic if the response of the sample was different from the response of the negative control with a certainty of 99%, based on a binomial distribution (see Appendix II).

2.4 Comparison experiment – part 2

In the second part of the comparison experiment, UV/H_2O_2-treated water was extracted with XAD-4 and XAD-2 + XAD-8 and tested on the comet assay.

2.4.1 Sampling

Water was sampled after MP UV/H_2O_2-treatment of sand-filtered water on 7 March 2011 at the pilot installation of Dunea at Bergambacht. The UV-reactor was set at 100% power, H_2O_2 was dosed at 10 mg/L H_2O_2. Four extensively cleaned 35-L stainless steel barrels were filled, after which 10,5 g Na_2SO_3 was added to each barrel to quench the residual H_2O_2. The barrels were transported to KWR and kept at room temperature, to ensure these large volumes of water had reached the required room temperature at the time of extraction the following day.

2.4.2 Sample extraction with XAD-4 and XAD-2 + XAD-8 and concentration

50 L of the sampled water was extracted with XAD-4 as described in 2.3.2, together with a procedure control. These yielded extracts of 2 mL in ethanol (25,000-fold concentrated).
Another 50 L of the water was extracted with XAD-2 in series with XAD-8, following the EPA protocol used by Kashinkunti *et al*. (WQTC 2009) and kindly provided by Debbie Metz (Greater Cincinnatti Water Works, U.S.A.), with some modifications.
XAD-2 and XAD-8 (both from Rohm & Haas) were first purified as described for XAD-4 in SWI 88.111, except that for an acrylate resin like XAD-8, the purification steps with lye are omitted. Then, a column with 50 mL of XAD-2 and one with 50 mL of XAD-8, was prepared. 50 L of the sampled water was acidified to 0,02 N HCl (pH 2) with hydrochloric acid and then passed first through the column with XAD-8 and then through the column with XAD-2 at 50 mL/min (overnight). Both columns were eluted separately with 200 mL ethylacetate. The water layer on the bottom was tapped of in a separation funnel, then the two extracts were combined. In contrast to the protocol, the ethylacetate was not dried with sodium sulphate, because extracted compounds may be trapped in the sodium sulphate crystals and be lost for analysis.Instead, the remaining water was removed by azeotropic distillation at 250 mbar and 40 °C. The ethylacetate was evaporated with a Rotavapor EL 130 of Büchi, in combination with a PVK 700 Vacume controller of MLT AG Labor-Technik. The ~10mL of remaining sample was transferred to a pre-weighed tube. The 1L round bottom flask was rinsed with a minimum amount of ethyl acetate to assure negligible loss of sample. The sample was "blown down" with nitrogen until slightly less than 2 mL. The volume was replenished with ethylacetate to 2 mL (25,000-fold concentrated) by weight.

2.4.3 Comet assay

The comet assays was performed by TNO. First, a neutral red uptake assay was performed as described in Borenfreund and Puerner (1985) with minor modifications, to check for cytotoxicity on the HepG2 cells (obtained from dr. B. Knowles). The HepG2 cells were treated for 3h with 0.25%, 0.5% and 1% of the water extracts in HBSS (v/v). 1% Triton X-100 (v/v) was used as positive control for cytotoxicity. Details can be found in Appendix III.
The comet assay was performed as described by Singh *et al.* (1988), with minor modifications as fully described in Appendix III. In brief, HepG2 cells were treated both for 3 h and for 24 h with HBSS medium containing aliquots of water extract at a concentration of 1% (v/v) in duplicate (exposure to a 200-fold concentration of the water samples). 25 µg/mL methyl methane sulfonate and 50 µg/mL benzo[a]pyrene in DMSO were used as positive controls for genotoxicity, respectively.

DNA damage was evaluated by calculation of the mean %tail DNA for a total of 200 cells per water sample (50 cells per slide, two slides per culture and two cultures per water sample). The water extracts were considered positive for genotoxicity when a three-fold increase in tail intensity over the negative control was observed. In addition to the prior neutral red uptake assay, viability was also checked by registering the number of ghost cells, though excluding them from the genotoxicity analysis. The relative proportion of ghost cells had to be less than 30%.

3 Results and discussion

3.1 Results preparative experiment 1

The results of the first preparative experiment are given in Figure 1 and Appendix IV.

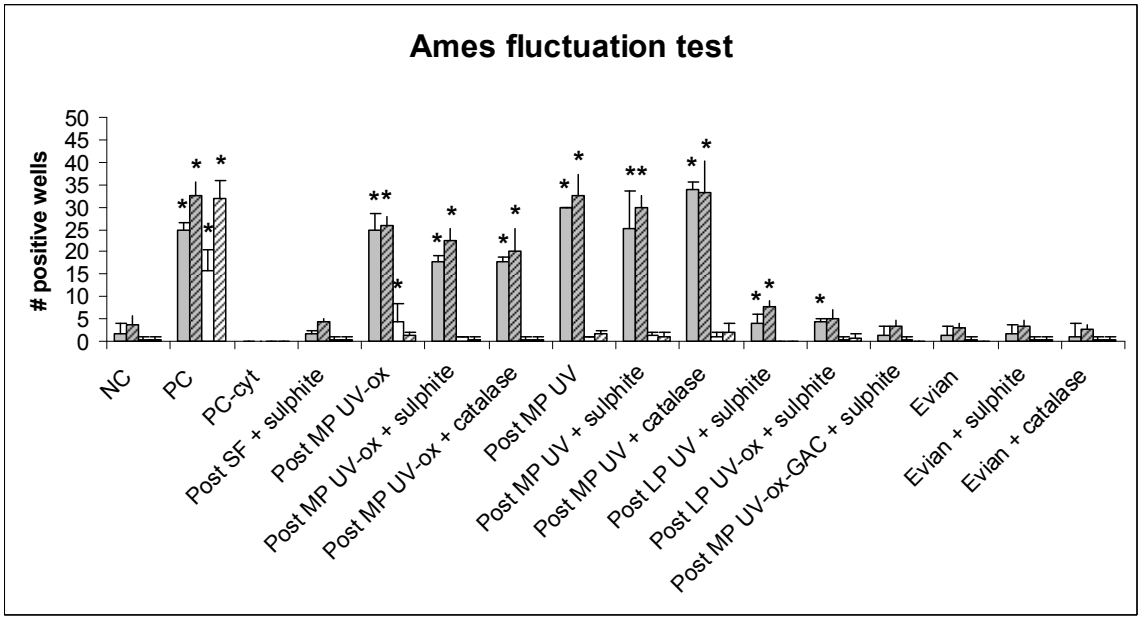

Figure 1. Ames fluctuation test results for different water extracts, to which either sulphite, catalase or nothing was added to neutralize residual H_2O_2, tested in TA98 (grey bars) and TAMix (white bars), with (striped bars) and without (solid bars) S9. NC = negative control, PC = positive control, PC-cyt = positive control for cytotoxicity, SF= sand filtration, MP = medium pressure, LP = low pressure, UV-ox = UV +H_2O_2. Error bars denote the standard deviation (n= 3). Asterisks denote responses determined to show genotoxicity, i.e. deviating form the NC with 99% certainty.

All controls were good, although the TA98-S9 test was repeated because of a somewhat high background in the initial test (2.7 yellow wells average). In comparison to this initial test, in the repeated test, with a lower background of 1.7 yellow wells average, three samples were not found to be genotoxic: the water post SF, the water post GAC and the Evian. All other samples gave the same final outcome in both tests. In the tests with S9, all samples were found to be cytotoxic (see Appendix IV), for an unknown reason. This effect of the S9 had not been observed before and could not be reproduced. This did imply that the level of genotoxicity seen in these tests could have been lowered by the impaired growth of the bacteria. This does not seem to be the case in TA98 + S9, however, as the responses there were mostly slightly higher than in TA98-S9, while in Heringa *et al.*(2011), the response in TA98+S9 was always lower than in TA98-S9.

The MP UV/H_2O_2-treatment in the pilot plant of Dunea clearly caused a formation of genotoxic activity in TA98, not in TAMix, which was removed again by GAC, as seen in Heringa *et al.* (2011). This formation and removal has thus been observed in four different treatment plants, treating three different water sources. Treatment with LP lamps caused only slight genotoxicity, clearly much less than with MP lamps. The main difference between the MP and LP lamps is the spectrum of emitted UV-light. As a result, photo-induced processes are a far more important process for MP lamps than for LP lamps. As first postulated by Heringa et al. (2011), these results are another indication that photo-induced processes play an important role in the formation of the genotoxic by-products. Another difference between the lamps under the test conditions of this experiment were the UV doses achieved: 875 mJ/cm2 (MP lamps), 741 mJ/cm2 (LP lamps) (Lekkerkerker *et al.*, accepted). Both the spectral differences and the

different UV doses achieved by the three lamp types could explain the observed differences in genotoxicity formation.
The genotoxic activity was slightly higher in the absence of oxidation by H_2O_2 (Post MP UV), but the difference with water post MP UV/ H_2O_2 was less than seen in Heringa *et al*. (2011). These results confirm the previous observation that the genotoxic activity is caused by the UV-light and not the added peroxide. It seems the peroxide can decrease the formed genotoxic activity, but to differing degrees. It is unknown on what factors the peroxide effectivity in decreasing the genotoxic activity is dependent; this would need to be further investigated. Possibly, different genotoxic compounds are formed in different water types, which are differently transformed by the OH-radicals produced from the peroxide.
No effect of the addition of neutralizing agent was observed. Heringa *et al*. (2011) already showed that sulphite was not extracted from the water and did not have an effect on the genotoxicity of sand-filtered surface water, but had not tested the effect of sulphite on UV/ H_2O_2-treated water. Possibly, the compounds in this treated water could react with the sulphite to form genotoxic substances. The present results show the genotoxic activity is not caused by the addition of sulphite. Residual H_2O_2 and catalase also do not affect the response of the Ames fluctuation test.

The MP UV/H_2O_2-treated water from the pilot plant of Dunea was thus found to be suitable for the desired comparison test and the choice of neutralization agent appeared not to be of importance.

3.2 Results preparative experiment 2

The results of the second preparative experiment are given in Figure 2 and in Appendix V.

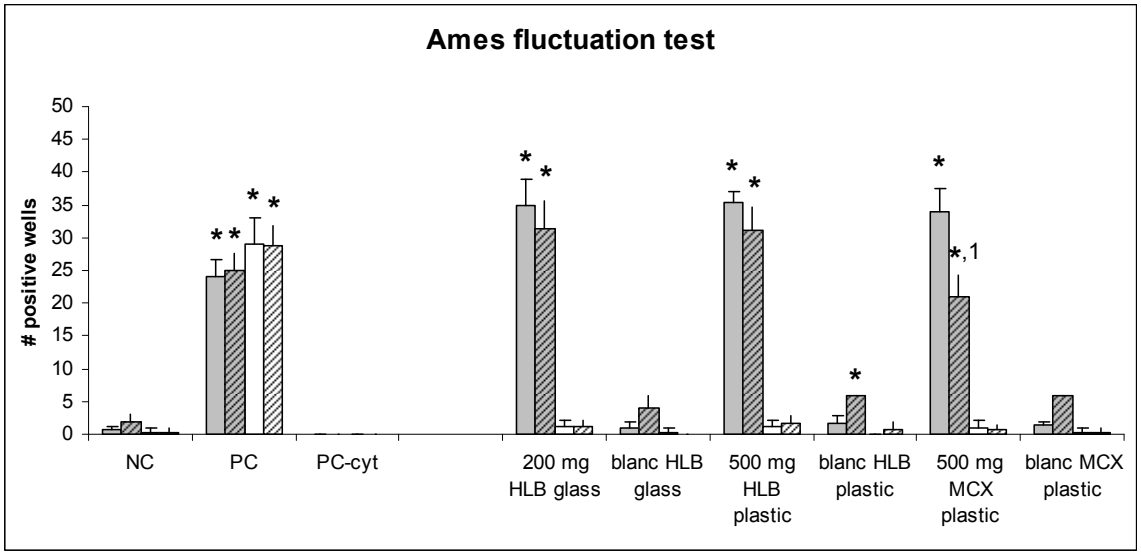

Figure 2. Ames fluctuation test results for UV /H_2O_2-treated water and blancs (no water), extracted with three different SPE materials, tested in TA98 (grey bars) and TAMix (white bars), with (striped bars) and without (solid bars) S9. Error bars denote the standard deviation (n= 5 x 3 for the water extractions, n = 1x 3 for the blancs). Asterisks denote responses determined to show genotoxicity, i.e. deviating form the NC with 99% certainty.1 = a significantly different response from those of the other materials (p <0.001).

The responses of the extracts from Oasis® HLB in glass and in plastic were found to be the same, but in TA98+S9, the response of the extract from Oasis® MCX was significantly different from those of the two Oasis® HLB extracts. Therefore, Oasis® HLB was chosen for the comparison experiment. The blanc extract of Oasis® HLB in plastic showed a slight genotoxic response in TA98 +S9, which could indicate some contamination from this type of column. Using this type of column could therefore cause some artefactual increase in the response in TA98+S9. This procedure background was not seen in TA98 –S9, however, which was the main test of interest for UV /H_2O_2-treated water. As the practical advantages of Oasis® HLB in plastic, enabling automation and thus higher through-put, were large, and the possible

contamination from the plastic very limited, it was decided to use Oasis® HLB in plastic for the comparison experiment.

3.3 Comparison of XAD and Oasis HLB

The results of the genotoxicity analyses of the three different extracts in the Ames plate test are given in Figure 3 and in Appendix VI.

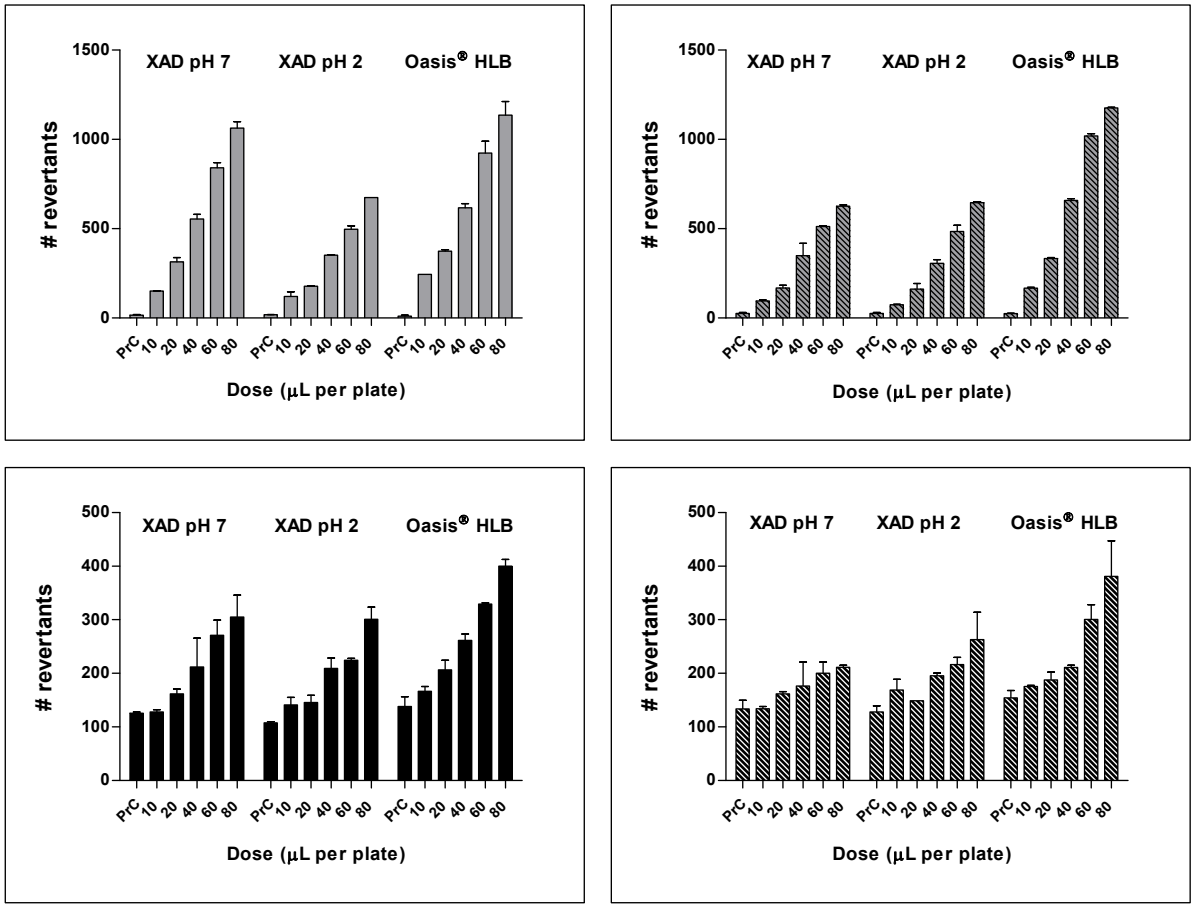

Figure 3. Responses in the Ames plate test of the three different extracts (XAD at neutral pH, XAD at pH 2, and Oasis® HLB in DMSO) of UV/H_2O_2- treated water, at different doses per plate, tested in TA98 –S9 (solid grey bars, top left), TA98 +S9 (striped grey bars, top right), TA100 –S9 (solid black bars, bottom left) and TA100 +S9 (striped black bars, bottom right). Error bars denote deviations of the mean (n=2), PrC = procedure control of corresponding extraction method.

By visual analysis, Figure 3 shows that in TA98 –S9, the Oasis® HLB and XAD pH 7 extracts give the same responses, while the XAD pH 2 extract gives a lower response. In the other three tests, both XAD extracts roughly give the same responses, while the Oasis® HLB extract gives a higher response. Altogether, Oasis® HLB extracts the genotoxic compounds in UV/H_2O_2- treated water best overall, but XAD, especially at pH 7, extracts these compounds quite well, too.

There is also a clear dose-response relationship for all extracts, proving that the response is not an artifact of the Ames plate test. The increase in response is smaller in TA100 than in TA98. It is remarkable that a response is seen in TA100, as all previous Ames tests with UV/H_2O_2- treated water did not show a response in TAMix, which detects the same type of mutations, namely base-pair substitutions. TA100 consists of one strain, with one particular base-pair substitution, and TAMix consists of six strains, with six different base-pair substitutions. If the compounds in this water only cause the one type of base-pair substitution detected with TA100, the number of revertants in TAMix will be 1/6th of that in TA100. This

1/6th of the response might well have been below the detection limit in TAMix. In the case of UV/H_2O_2-treated water, it appears that TA100 is the better strain to use.

The results of the genotoxicity analyses of the three different extracts in the Ames fluctuation test are given in Figure 4 and in Appendix VII. For this test, the volume of Oasis® HLB extracts appeared just insufficient, therefore in TA100 some dosages were not tested, or only with a single replicate. All controls were good. Some samples showed cytotoxicity, varying over the four tests, but never in a dose-related way or in a consistent pattern. Some individual responses may theoretically have been dampened by the decreased growth potential due to this cytotoxicity, but the overall patterns and conclusions are expected not to have been affected by this.

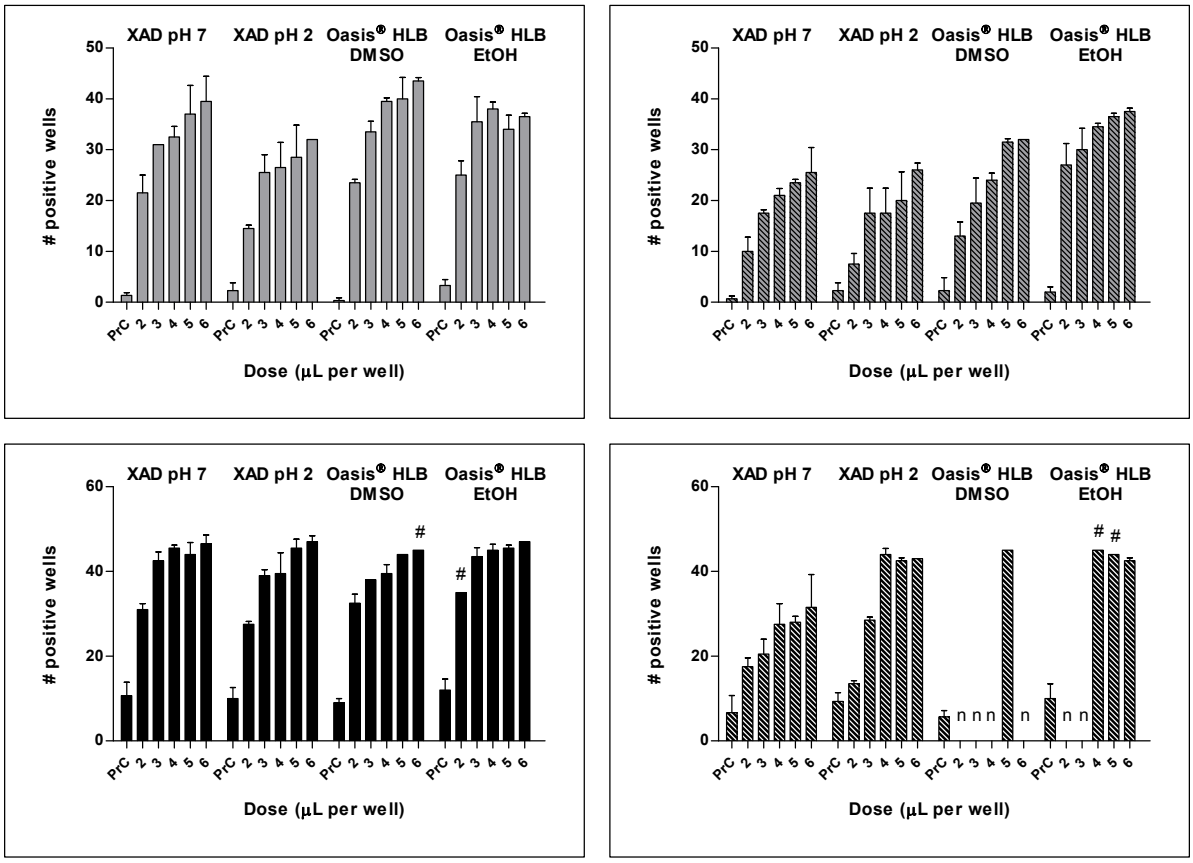

Figure 4. Responses in the Ames fluctuation test of the three different extracts (XAD at neutral pH, XAD at pH 2, Oasis® HLB in DMSO, and Oasis® HLB in ethanol) of UV/H_2O_2- treated water, at different doses per plate, tested in TA98 –S9 (solid grey bars, top left), TA98 +S9 (striped grey bars, top right), TA100 –S9 (solid black bars, bottom left) and TA100 +S9 (striped black bars, bottom right). Error bars denote deviations of the mean (n=2), #= single measurement (n=1), PrC = procedure control of corresponding extraction method, n = not tested by lack of sufficient extract.

BY visual analysis, Figure 4 shows that the Oasis® HLB in DMSO and XAD pH 7 extracts give the same responses in TA98 –S9, while the XAD pH 2 extract gives a lower response. In TA98 + S9, both XAD extracts roughly give the same responses, while the Oasis® HLB extract gives a higher response. In TA100 – S9 all extracts give the same responses, and in TA100 + S9, the XAD pH2 and Oasis® HLB in DMSO give similar responses, while the XAD pH 7 extract gives lower responses. Thus, also according to the Ames fluctuation test, Oasis® HLB extracts the genotoxic compounds in UV/H_2O_2- treated water best overall, but XAD extracts these compounds quite well, too.
Comparing the solvents DMSO and ethanol for the Oasis® HLB extracts, it can be seen that these do not give clear differences in the genotoxic response

There are clear dose-response relationships for all extracts, proving that the seen response is not an artifact of the Ames fluctuation test. The increase in response is smaller in TA100 than in TA98, mainly because the lowest dose already gives a high response and this test is limited by the maximum of 48 wells that can turn yellow. The dose-response lines in the fluctuation test are more variable than those in the plate test, caused by the lower numbers scored in the fluctuation test and the limit of 48 wells.

The extraction with XAD at pH 2 only occurs in series after the extraction at neutral pH, so less compounds may be left over to extract at pH 2. It is therefore remarkable that the pH 2 extract still causes a significant response in the Ames plate and fluctuation test, this indicates that there are other genotoxic compounds in the water than those extractable at neutral pH. There is possibility that at pH 2, the nitrite in UV/H_2O_2- treated water reacts with organic compounds (e.g. from the natural organic matter) to form new genotoxic compounds (e.g. Challis and Lawson, 1971;). It is well known that in the acid environment of the human stomach, nitrite (from nitrate of leafy vegetables) can react with amines (abundant in fish) to form the carcinogenic nitrosamines (de Kok and van Maanen, 2000). This suggests that the genotoxicity might not have been caused by the UV-treatment itself, but by the formed nitrite by the UV and the low pH of the extraction method. However, as similar genotoxic responses were observed at neutral pH (XAD pH 7), the occurrence of genotoxic compounds appears not to be due to the extraction method.

The results of the genotoxicity analyses of the three different extracts in the comet test are given in Figure 5 and in Appendix VIII (from TNO report V20022/01). This figure shows that none of the extracts gave a response in the comet assay, corresponding to the results of Heringa *et al*. (2011) with Oasis® HLB extracts only. XAD does not extract any new compounds that are genotoxic in the comet assay.

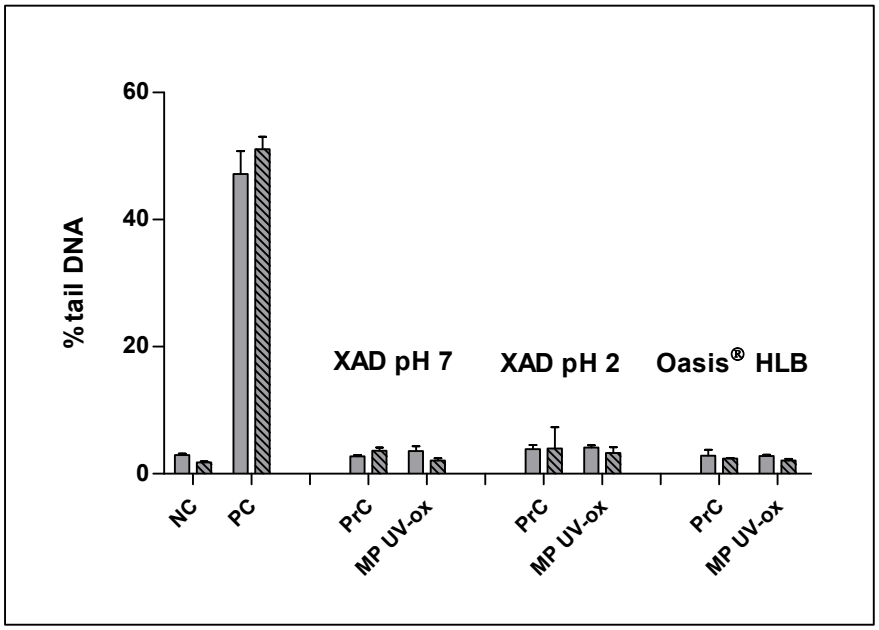

Figure 5. Responses in the comet test of the three different extracts (XAD at neutral pH, XAD at pH 2, and Oasis® HLB in DMSO) of UV/H_2O_2- treated water, incubated for 3h (solid grey bars and for 24 h (striped grey bars) in HepG2 human liver cells. Error bars denote standard deviations (n=200), NC = negative control, PC = positive control, and PrC = procedure control of corresponding extraction method.

Overall, it can be concluded that Oasis® HLB extracts the genotoxic compounds in UV/H_2O_2- treated water best, but XAD extracts these compounds quite well, too. The difference in extraction method can therefore not explain the different outcomes seen by Heringa *et al.* (2009) and Penders *et al*. (IUVA 2009).

3.4 Comparison of Ames plate test and Ames fluctuation test

Figure 3 and Figure 4 already show that both the Ames plate test and the Ames fluctuation test detect a dose-related genotoxic activity in the same extracts of UV/H_2O_2- treated water. Thus, qualitatively, the two test versions give a similar result. Umbuzeiro *et al*. (2010) also found that these two test versions produced similar results with TA98 +/- S9.
An effort was made to try and compare the two test versions quantitatively, too.

3.4.1 Quantitative translation of absolute responses

First, the absolute responses of the two test versions were compared to try and find a translation factor, with which the response of one version could be translated into the response of the other version. Figure 6 and Figure 7 show the responses in both test versions, set out on the same x-axis, with the dose expressed as litre equivalents (of the original water) added by the extract per mL of exposure medium (fluctuation test) or top agar (plate test), to enable comparison. It is thereby assumed that in the 90 minutes exposure, the compounds do not diffuse from the top agar (3.6 mL) to the bottom agar (20 mL) in the plate test, which would increase the volume and lower the relative dose.

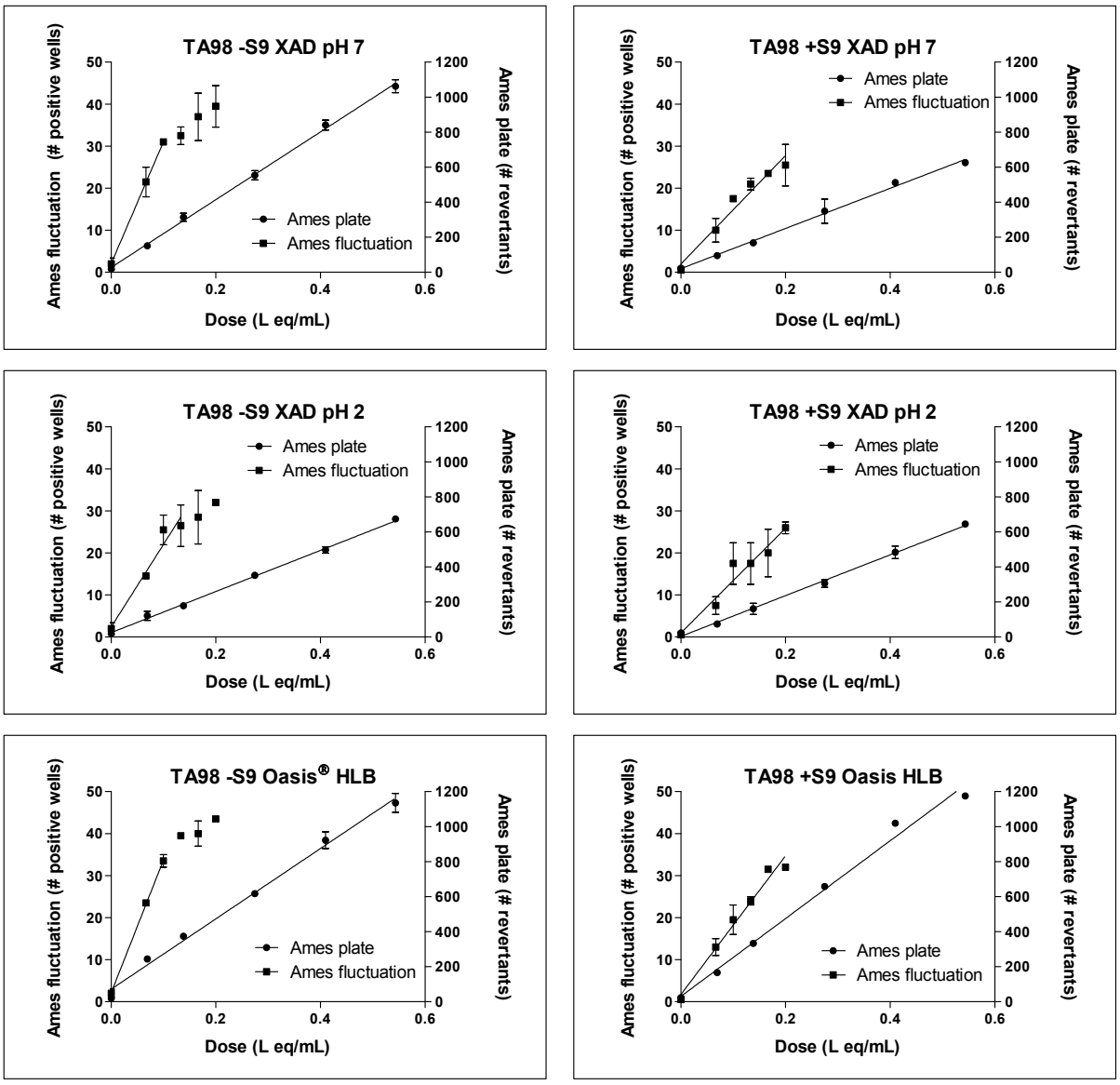

Figure 6. Responses of the Ames fluctuation test and Ames plate test for three different extracts of UV/H_2O_2-treated water, in TA98 +/- S9. The doses in the tests have been set as litre equivalents of water dosed per mL of culture medium (fluctuation test) of topagar (plate test), to enable easier comparison. Error bars denote standard deviations (n=2), linear regression lines were fitted through the data point (for the fluctuation tests data only in the visually linear range) .

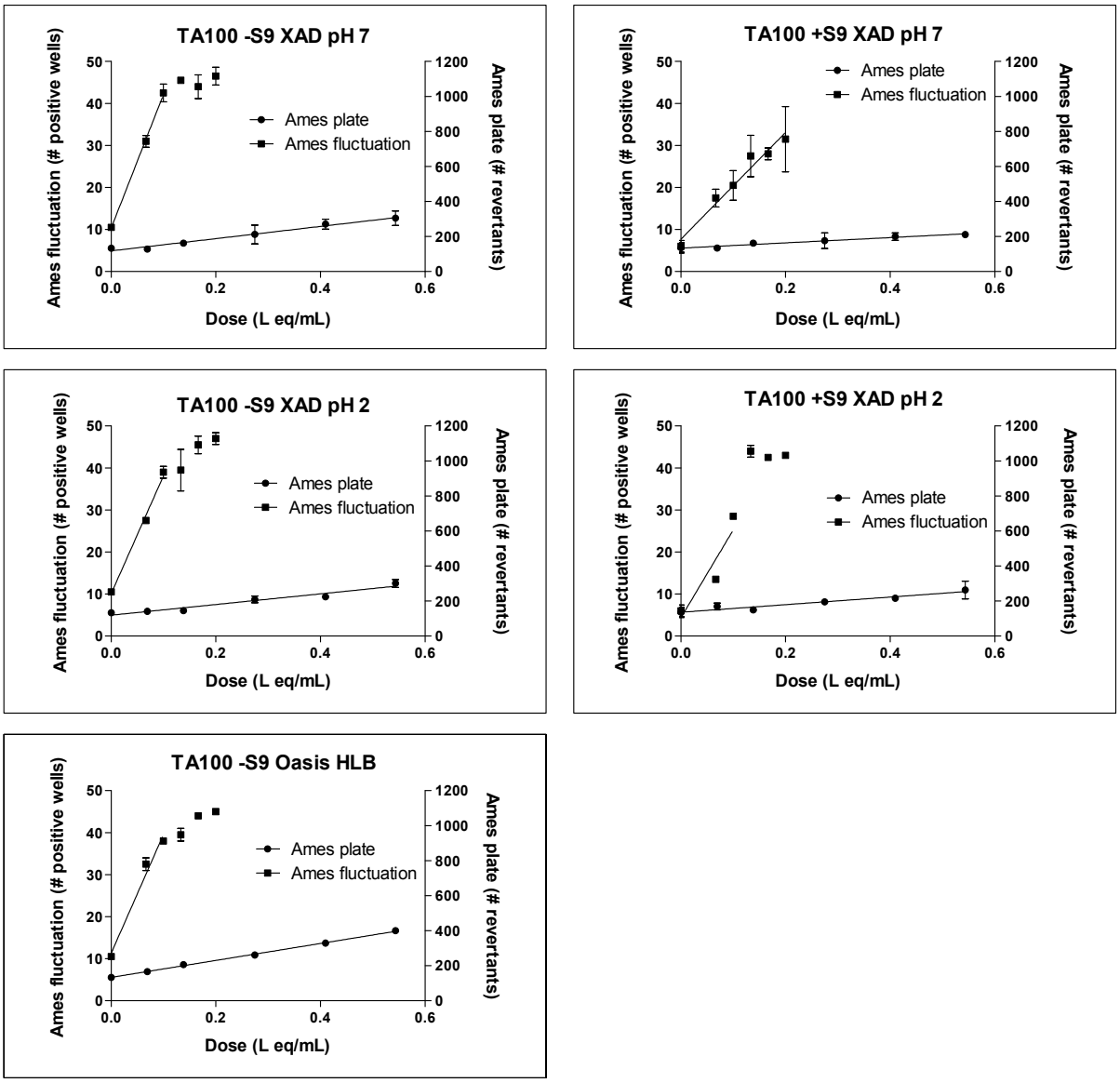

Figure 7. Responses of the Ames fluctuation test and Ames plate test for three different extracts of UV/H_2O_2-treated water, in TA100 +/- S9. The doses in the tests have been set as litre equivalents of water dosed per mL of culture medium (fluctuation test) of topagar (plate test), to enable easier comparison. Error bars denote standard deviations (n=2). There were too few data points for the Oasis® HLB extract in TA100 + S9 in the fluctuation test to make a comparison.

The concentration of bacteria in the topagar layer of the Ames plate test is the same as in the liquid exposure medium of the Ames fluctuation test: ~ 3 ×10^7 bacteria/mL. However, the volume of the topagar in the plate test is 3.61- 3.,68 mL (depending on how much sample is added), while the volume

of the liquid exposure medium in the fluctuation test is 300 µL: a factor 12 difference. The number of bacteria in the fluctuation test is therefore 12-fold lower than in the plate test. If the concentration of the chemical compound is the same, it could be expected that the number of mutations in the Ames plate test is 12-fold higher than that in the Ames fluctuation test. Of all revertant bacteria from the 90 minutes exposure, however, 80% (i.e. 48 * 50 µL = 2.4 mL of the 3.0 mL after addition of indicator medium) is divided over the 48 wells. It could therefore be expected that the number of mutations in the Ames plate test is (12/0.8=) 15-fold higher than that in the Ames fluctuation test.
As there were no data points at exactly the same dose of water, it could not be verified whether this theoretical factor of 15 is indeed found empirically. Therefore the slopes of the linear dose-response regression lines through the data points were compared.
It is clearly visible in the figures, and also logic, that there is a limitation in the translation of the slopes between the two test versions, because the Ames fluctuation test data are not fully linear. The Ames fluctuation test has a maximum response of 48 wells. The number of mutant bacteria is therefore only linearly related to the number of yellow wells at low number of mutant bacteria, because at high numbers, two or more mutant bacteria can be present in the same yellow well. The number of yellow wells will saturate from a certain number of mutant bacteria onwards. By calculation, this deviation from linearity starts to be significant (i.e. >5% chance of 2 or more mutants in a well) from 15-18 mutant bacteria onwards, so from 15-18 yellow wells onwards. Officially, a linear regression line through the Ames fluctuation test data can then only be drawn up to 15-18 yellow wells. The data produced here, however, include insufficient data points within this range. Therefore, a linear regression line was drawn up to the points that visually started to deviate from linearity. The slopes of the obtained regression lines with both test versions, and their ratios, are given in Table 4. In TA98, the slope ratios vary between 6 and 9, with an outlier at 13. This is comparable to the average slope ratio of 6.9 (i.e. $10^{0.84}$) found by Umbuzeiro *et al.* (2010) in TA98 +/- S9. In TA100, the slope ratios vary between 1 and 2. The reason of the outlier and for the remarkable difference between TA98 and TA100 is unclear. It must be noted that the regression lines were drawn through a very minimal number of data points from the Ames fluctuation test, thus the given slopes from the fluctuation test and the ration of the slopes have limited reliability. These results are only indicative.

Table 4. Slopes of the linear dose-response regression lines of three different extracts of UV/H_2O_2- treated water in the Ames plate test and the Ames fluctuation test, and the ratio of these slopes.

Strain	Extract	Slope Ames plate	stdev	Slope Ames fluctuation	stdev	Ratio slopes tests	stdev
TA98 -S9	XAD pH 7	1935	37	290	19	6,67	0,46
	XAD pH 2	1174	26	197	19	5,96	0,59
	Oasis® HLB	2009	73	316	13	6,36	0,35
TA98 +S9	XAD pH 7	1147	41	129	12	8,89	0,89
	XAD pH 2	1164	32	125	16	9,31	1,22
	Oasis® HLB	2222	71	165	11	13,47	1,00
TA100 -S9	XAD pH 7	349	39	318	14	1,10	0,13
	XAD pH 2	305	28	281	14	1,09	0,11
	Oasis® HLB	484	14	283	23	1,71	0,15
TA100 +S9	XAD pH 7	151	29	127	16	1,19	0,27
	XAD pH 2	217	36	209	43	1,04	0,27

In any case, the experimentally determined factor of difference is (much) lower than the theoretical factor of 15, meaning the number of revertants in the plate test are lower than assumed, or the number of yellow wells in the fluctuation test higher than assumed. This might be explained by a factor not taken into account so far: the rate of diffusion of chemicals through agar is much slower than through fluid medium, especially for larger molecules (see Figure 8). Chemicals will therefore have less chance of encountering the bacteria and their DNA in agar, than in fluid medium, leading to fewer revertants in the Ames plate test and thus to a lower translation factor. Perhaps, the compounds in UV/H_2O_2- treated water that cause the mutations in TA100 are larger than those causing mutations in TA98, leading to

even the observed even lower translation factor. This possible explanation could be verified with comparative experiments with compounds of different sizes.
With the present set of data, the obtained factors of difference should only be used for UV/H_2O_2- treated water, as the compounds, and thus the diffusion rates, and thus the level of genotoxicity in the Ames plate test, can be different in other samples. And even with UV/H_2O_2- treated water, the range of ratios should be used, to obtain a range of possible responses in the other version of the test, showing the uncertainty in such a translation.
The number of revertants in the plate test at a 1-L equivalent dose can then be calculated from the number of yellow wells in the Ames fluctuation test at the highest dose (6 µL extract, i.e. 60-mLequivalent) with equation 1, where Y is the number of yellow wells, R is the number of revertants counted, R_b is the background number of revertants, sf is the factor difference in the slopes of the dose-response lines and Y_b is the background number of yellow wells. According to Table 4, sf ranges between 6 and 13 in TA98, and in TA100, sf is 1-2. The factor of 0.73 corrects for the difference in compound concentration at a 1-L equivalent dose in the Ames plate test and the usual 60-mL equivalent in the Ames fluctuation test.

$$R = (Y - Y_b) \cdot \frac{sf}{0.73} + R_b \qquad \text{equation 1}$$

It must be noted that this translation method has not been validated with a validation set of data yet, as such a set of data is not available at present. This is recommended for future work. In the mean time, this translation method must be used with caution.

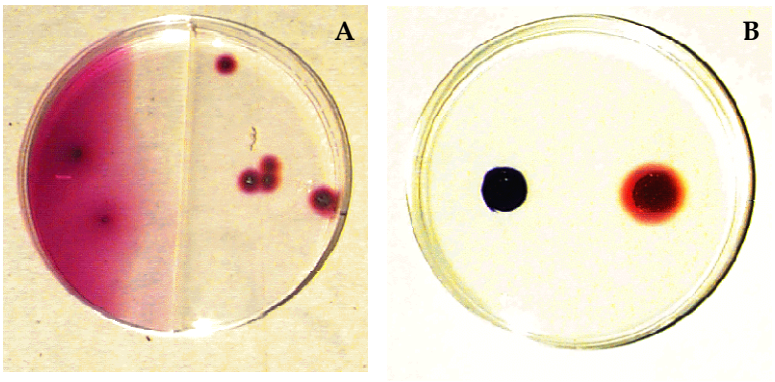

Figure 8. Diffusion of chemicals through agar: potassium permanganate diffuses faster through distilled wate in left half of the petri dish than on the right half (A) and the larger molecules of methylene blue on the left diffuse a lot slower through agar than the small molecules of potassium permanganate (B). From the website of the Henry Ford Community College, http://sciweb.hfcc.net/biology/jacobs/bio131/diffusion/diff&os.html.

3.4.2 Comparison of responses above threshold of concern

Umbuzeiro *et al*. (2010) made a quantitative comparison by comparing the lowest doses that provided a positive response in each assay. They found that these were similar. Such a comparison was not informative with the data produced in the present work, as all doses were positive in both assay versions in TA98 +/- S9 and in the Ames fluctuation test with TA100 +/- S9, or all negative in the Ames plate test with TA100 +/- S9.
As a second exercise, therefore, the responses in the Ames plate and Ames fluctuation test were instead compared to the threshold of significance of each version, to compare the final conclusions about the genotoxicity of the water.

In the Ames plate test, the threshold of significance traditionally employed by KWR is 3-fold the background response (number of spontaneous revertants): the number of induced revertants (i.e. with the background subtracted) must be twice the background to call a response genotoxic. For a sample to be called genotoxic, KWR employed the rule that the response at a 1-L equivalent dose must be genotoxic. Figure 9 shows the responses of the three prepared extracts of the water at this 1-L equivalent per plate (i.e. 40 µL) dose, as well as the 3-fold threshold.
Clearly, the responses in TA98 +/- S9 are well above this threshold of significance, thus this water is called genotoxic according to the traditional rule. In TA100, the responses of the samples do not exceed the threshold, but the positive controls also do not. The positive control values of TA100 –S9 and TA100 +S9 were within the 2S range of historical data, however, and thus accepted.
The threshold of significance is also very high in TA100, much higher than in TA98, making it difficult for a sample or a positive control to exceed this threshold. This makes the TA100 much more insensitive in detecting a genotoxic activity than TA98. The cause of this much higher detection limit line is the high rate of spontaneous revertants in the TA100 strain, resulting in a high response of the negative control. When applying a statistical method which multiplies this background by three to obtain a detection limit, only very high sample responses can result in a genotoxicity label. The 3-fold threshold applied by KWR until now is actually quite conservative compared to the multiplication by two applied by e.g. TNO and Haider *et al*. (2002). Even this multiplication by two is found to be too conservative by statisticians, though it is the most widely used method worldwide (e.g. Kim and Margolin, 1999). Other evaluation methods have been proposed, and it is recommended that for further classic Ames tests, the evaluation method at KWR is improved.

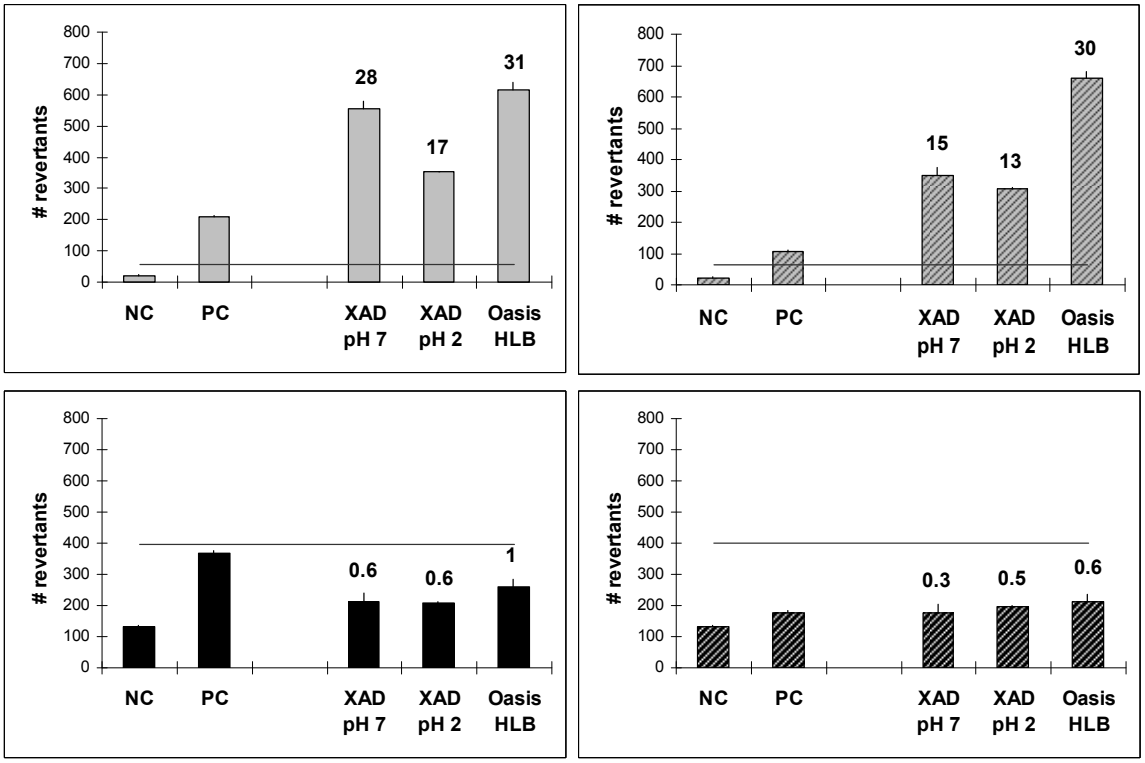

Figure 9. Absolute responses of three different extracts of UV/H_2O_2- treated water in the Ames plate test at 1-L equivalent doses per plate, in TA98 –S9 (solid grey bars, top left, TA98 +S9 (striped grey bars, top right), TA100 –S9 (solid black bars), and TA100 +S9 (striped black bars). Error bars denote standard deviations (n=2), NC = negative control, PC = positive control, the line indicates the threshold of significance (3-fold NC), and the numbers above the sample bars give the induction factors.

Figure 9 also gives the induction factors of the sample responses, calculated by subtracting the background from the sample response and then dividing by the background, as performed by KWR traditionally. The induction factors in TA98 are very high; a quick scan through the Ames plate test

reports of KWR showed that no such induction factors have been found before, even in chlorinated surface water.

For the Ames fluctuation test results, the method is based on the binomial distribution of the data, providing a sound theoretical basis. A response is called genotoxic if the threshold for significance with 99% certainty is exceeded. A water sample is called genotoxic if the response at the usual highest dose of 6 µL extract (a 60-mL equivalent of the original water) gives such a genotoxic response.
Figure 10 shows the responses of the three prepared extracts of the water at the highest applied dose (6 µL extract, a 60-mL equivalent of the original water), as well as the statistical threshold of significance, based on the binomial distribution. All extracts exceed this threshold in all four tests, this water is therefore considered to be genotoxic.

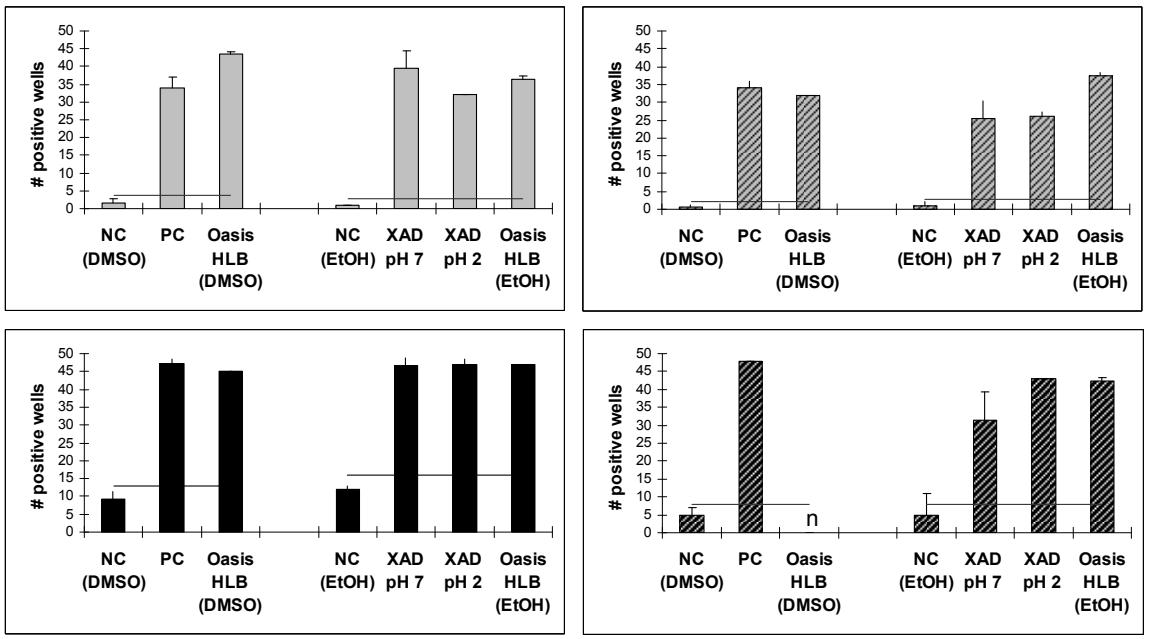

Figure 10. Absolute responses of three different extracts of UV/H_2O_2- treated water in the Ames fluctuation test at 60-mL equivalent doses per well, in TA98 –S9 (solid grey bars, top left, TA98 +S9 (striped grey bars, top right), TA100 –S9 (solid black bars), and TA100 +S9 (striped black bars). Error bars denote standard deviations (n=2), NC = negative control, PC = positive control, EtOH = ethanol, and the lines indicate the threshold of significance (99% certainty of deviation form NC), differing per applied solvent.

In contrast to the Ames plate test, the Ames fluctuation test does detect genotoxic responses in the TA100 strain. In the Ames fluctuation test, the background response is higher in TA100 than in TA98, too, but with the different statistical method applied, this does not lead to an unpassable detection limit.

It may be questioned why the doses of 1-L water equivalent in the Ames plate test and 60-mL water equivalent are chosen as reference doses, to determine whether a water sample is genotoxic. The 1-L equivalent was chosen by expert gut feeling, also based on the fact that people in the Netherlands drink 1-2 L of tap water (including drinks prepared from tap water) a day. The 60-mL dose in the fluctuation test was chosen for practical reasons: 6 µL was the highest dose of extract in organic solvent that should be added to the medium, as this gives a concentration of 2% of organic solvent. The concentration factor of 10,000 was the highest obtainable from 1 L of water when needing 100 µL for the four tests.
Ideally, however, this reference dose is chosen by a relation to human health risk. Until recently, this was not possible, as a complex mixture of mostly unknown compounds and concentrations is present in the water, each posing different risks. Now, a limited relation to human health risk can be made: comparing the effect at a certain water dose to a limit below which no effects can reasonably be expected and above which further study would be necessary. This limit is called the Threshold of Toxicological Concern (TTC; Kroes *et al.*, 2004). This is a dose, or concentration, below which no compound will have a toxicological effect. For genotoxic substances, the TTC for drinking water has been determined to be 10 ng/L for an individual compound. Water with a genotoxic compound present below this level will not pose an unacceptable cancer risk from this compound. Above this level, a compound may still have no health effect up to a certain, higher concentration, but unacceptable risks can not be excluded without knowing more about the specific compound. The TTC is thus a conservative value (Schriks *et al.*, 2010). This TTC for individual compounds can be used to derive a TTC for the response in the Ames test. The TTC is based on the risks posed by the most potent genotoxic compounds. When one of the most potent genotoxic compounds is tested on the Ames (fluctuation) test in an extract representing a water concentration of 10 ng/L, it will give a certain response. This response can be seen as a genotoxicity response-TTC. When a water extract of unknown composition gives a response below this genotoxicity response-TTC in the same test, it can be assumed that this water poses no health risks as the final risk

(sum of potency*dose) of the present compounds is lower than that of 10 ng/L of a very potent compound.
In the Ames fluctuation test, the lowest concentrations of the most potent compounds found so far, 4-nitroquinolineoxide (4-NQO) in absence of S9 and 2-aminoanthracene (2-AA) in presence of S9, that give a detectable response, are given in Table 5. For strain TA100, such data are not available yet. Clearly, 10 ng/L of these compounds can not be detected yet at the chosen reference dose of 6 µL extract, thus any response in the Ames fluctuation test is above the response-TTC. For any sample for which a response is found in the Ames fluctuation test, health risks can therefore not be excluded. It is very well possible then, that the present compounds do not pose a risk, because they are not well absorbed in the gut, for example, but this then remains to be investigated. For a sample for which a response is below the detection limit, a genotoxic constituent may still be present just above 10 ng/L, so health risks can also not be fully excluded, but chances are small they will occur.

Table 5. Detection limits of the most potent compounds found so far in the Ames fluctuation test, i.e. the lowest concentrations of these compounds producing a detectable effect when dosed at 6 µL of extract. Determined from dose-response relationships

Test	**$DL_{extract}$**	**DL_{water} (extract 10.000-fold concentrated)**
TA98 –S9	~ 2 mg/L 4-NQO	~ 200 ng/L 4-NQO
TA98 +S9	~ 2.5 mg/L 2-AA	~ 250 ng/L 2-AA
TAMix –S9	~ 0.5 mg /L 4-NQO	~ 50 ng/L 4-NQO
TAMix +S9	~ 10 mg/L 2-AA	~ 1000 ng/L 2-AA

In the Ames plate test as performed at KWR, dose-response curves for potent genotoxic compounds could not be found in the archives as they probably have been determined too long ago. Literature data cannot be used as the exact test method often differs in details from that of KWR. So far, new curves have only been determined for 4-NQO in TA98 –S9.There, the detection limit is 18 ng of 4-NQO per plate, which corresponds to a water concentration of 18 ng/L when an equivalent of 1 L is dosed. This is still higher than 10 ng/L. Thus, when no genotoxic response is detected at a 1 L-equivalent dose, a compound may still be present at a concentration posing health risks, and thus, a health can not be excluded. Only if no genotoxic response is detected at twice this dose (a 2-L equivalent), health risks can be excluded because then the response is equivalent to that of potent genotoxic compound at 9 ng/L.
In this study, both in the Ames fluctuation test and in the plate test, genotoxic responses were detected in TA98 (the strain where some reference data were available) at the employed reference doses, thus health risks cannot be excluded when this UV/H_2O_2- treated water would be directly consumed life-long. Both test versions agree on this conclusion.

In summary, the Ames plate test and the Ames fluctuation test both detected a dose-related response in extracts of the UV/H_2O_2- treated water, which was above the threshold of significance at the employed reference doses in both test versions. Based on the TTC, in both test versions health risks cannot be excluded for water producing such a response. The genotoxic compounds might still not pose a health risk, for example because they are not absorbed in the gut, but this needs to be further investigated first. The two test versions therefore appear to agree well in the analysis of this UV/H_2O_2- treated water, which does not provide an explanation for the different results found by Heringa *et al.* (2011) and Penders *et al.* (IUVA 2009).

3.5 Comparison of XAD-4 and XAD-2 + XAD-8 on the comet assay

The results of the genotoxicity analyses of the different types of XAD extracts in the comet test are given in Figure 11 and in Appendix VIII (from TNO report V20022/01). This figure shows that none of the extracts gave a response in the comet assay. The absence of response of the XAD-4 extracts corresponds to the results of the earlier XAD-4 extracts of water of the same source, but from an earlier data (see Figure 5). The absence of response from the extract of XAD-2 and XAD-8 does not correspond to the results of Kashinkunti *et al*. (WQTC 2009), however. They did find a response in such extracts in the comet assay. However, their comet assay was performed in Chinese hamster ovary (CHO) cells, while HepG2 liver cells were used in the present study. Liver cells contain more metabolizing enzymes than ovary cells, which can detoxify compounds. However, an exposure time of 3 h was specifically employed to detect any damage caused before metabolization of genotoxic compounds. With no other relevant differences known between these cell types, it is not expected that this difference in cell type can have led to large differences in detected DNA damage. It cannot be ruled out, however. Also, small differences in how the extraction was performed at the two different laboratories could be responsible, even though care was taken to follow the same procedure as applied by Kashinkunti *et al*. Further in-depth comparisons would be necessary to find an explanation for the differences found by Heringa *et al*. (2011) and Kashinkunti *et al*. (WQTC 2009). For example, leftover XAD extracts could be tested in the comet assay by the laboratory employed by Kashinkunti et al. As a new positive result in the comet assay does not add much to the hazard posed by UV/H_2O_2- treated water, as detected by the Ames tests already, it is doubtful whether such additional studies on the comet assay are worthwhile.

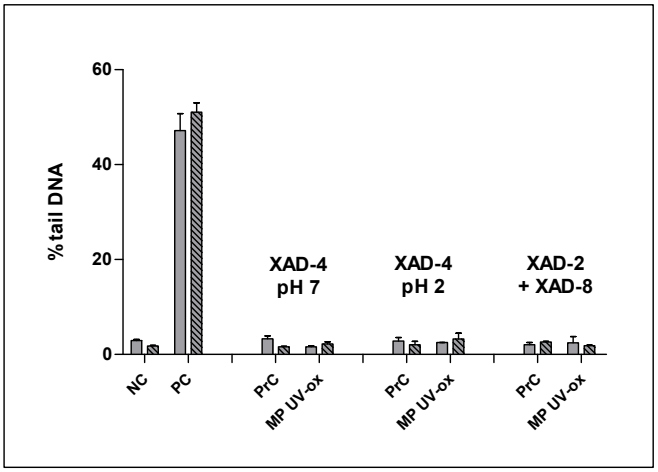

Figure 11. Responses in the comet test of the three different extracts (XAD-4 at neutral pH, XAD-4 at pH 2, and XAD-2 + XAD-8) of UV/H_2O_2- treated water, incubated for 3h (solid grey bars and for 24 h (striped grey bars) in HepG2 human liver cells. Error bars denote standard deviations (n=200), NC = negative control, PC = positive control, and PrC = procedure control of corresponding extraction method.

In summary, no genotoxic responses were detected in the XAD-4 and XAD-2 + XAD-8 extracts of the UV/H_2O_2- treated water in the comet assay. This provides no explanation for the differences found by Heringa *et al*. (2011) and Kashinkunti *et al*. (WQTC 2009).

3.6 Comparison of coconut charcoal and Oasis® HLB on the Ames fluctuation test

The results of the genotoxicity analyses of the three different extracts in the Ames fluctuation test are given in Figure 12 and in Appendix VII. For this test, the volume of extracts appeared just insufficient,

therefore in TA100 some dosages were not tested, or only with a single replicate. All controls were good. Quite some samples showed cytotoxicity, varying over the four tests, but never in a dose-related way or in a consistent pattern. Some individual responses may theoretically have been dampened by the decreased growth potential due to this cytotoxicity, but the overall patterns and conclusions are expected not to have been affected by this.

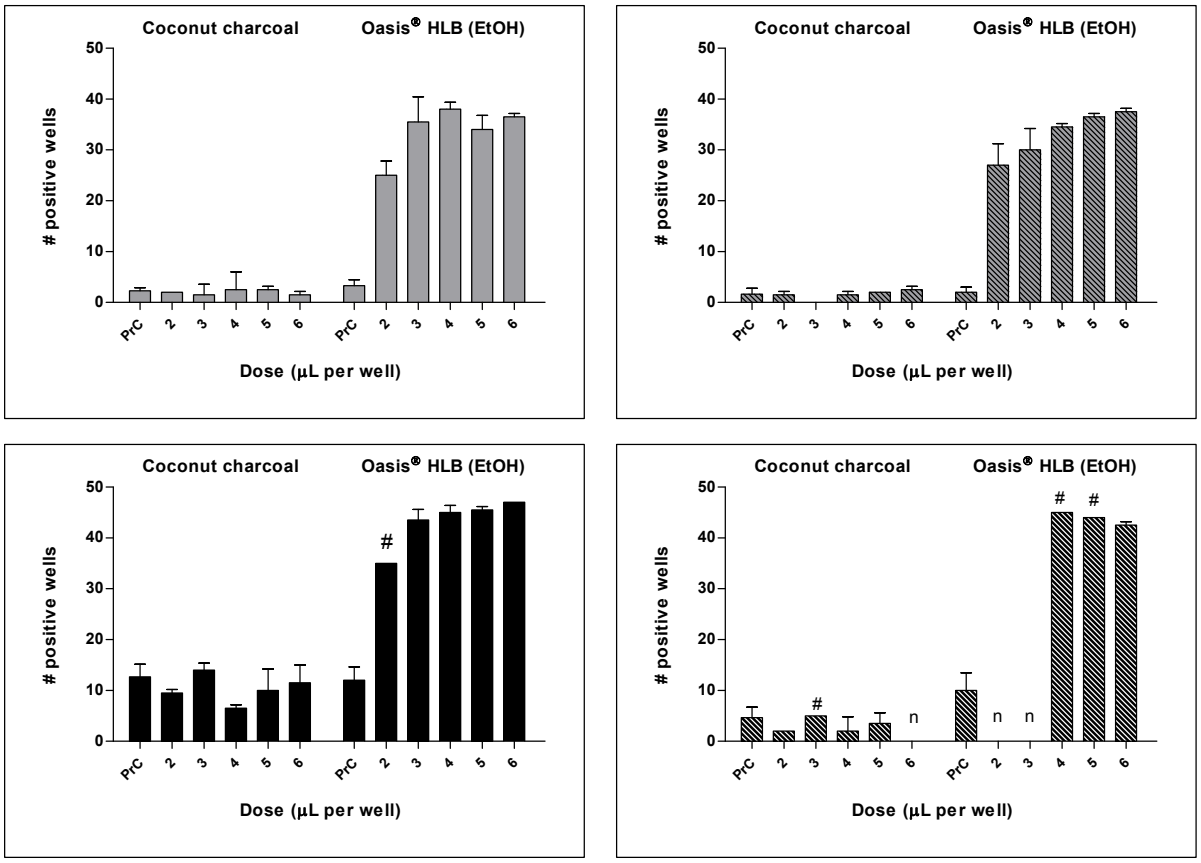

Figure 12 Responses in the Ames fluctuation test of the Oasis® HLB and coconut charcoal extracts (both in ethanol) of UV/H_2O_2- treated water, at different doses per plate, tested in TA98 –S9 (solid grey bars, top left), TA98 +S9 (striped grey bars, top right), TA100 –S9 (solid black bars, bottom left) and TA100 +S9 (striped black bars, bottom right). Error bars denote standard deviations (n=2, except where indicated with #, where n=1), PrC = procedure control of corresponding extraction method, n = not tested by lack of sufficient extract.

Coconut charcoal extracts gave no genotoxic response in the Ames fluctuation assay, this material apparently does not extract any of the genotoxic compound(s) from the UV/H_2O_2- treated water. No nitrosamines are thus missed by the Oasis® HLB extraction, also indicating there are no detectable levels of hydrophilic nitrosamines in the tested water, and such compounds are thus not responsible for the observed genotoxicity. The compounds that are responsible for the genotoxicity apparently either do not adsorb well to the coconut charcoal, or they adsorb so well they are not eluted. These results are in contrast to the observation in Figure 1 and in Heringa *et al*. (2011), where GAC was found to remove the formed genotoxic substances well. Explanations might be the stated strong adsorption which blocks elution, or that GAC removes the compounds by another process than adsorption (e.g. microbial degradation), or some specific difference between the adsorptive properties of coconut charcoal and the carbon used in the GAC of the analyzed setups. The second explanation also matches with the finding in Heringa *et al*. (2011) that even very old GAC still removed the genotoxic by-products well: the adsorptive capacity will have been minimal then, but the microbial degradation would have been up to speed.

In summary, the coconut charcoal extract did not cause any genotoxic response, in contrast to the Oasis® HLB extract. This indicates the observed genotoxicity in Oasis® HLB extracts is not caused by hydrophilic nitrosamines. These results might also be an indication that the genotoxic substances in UV/H_2O_2- treated water are not removed by adsorption in GAC, but by another process, e.g. microbial degradation.

3.7 Choice of methods

The Ames fluctuation test has the advantages of needing less sample, needing less work to perform and enabling automation due to the multi-well plate format. Because less sample is needed, the smaller water samples from laboratory test setups (e.g. 0.5 L of water) can be analyzed, which are not sufficient for the Ames plate test (needing at least 12 L of water). Smaller sample sizes enable the use of commercial SPE cartridges for extraction of the water, which can typically take up to 2 L of water. With these commercial SPE cartridges (e.g. with Oasis® HLB), less work is necessary for the extraction of water than with an XAD-extraction, as the XAD needs to be purified first and large glass setups need to be manually cleaned and put up for the extraction with XAD. Additionally, because XAD extraction needs such large setups, the limited laboratory space only allows a limited number of samples to be extracted simultaneously (e.g. 3 at KWR). With the commercial SPE cartridges, 16 of even 32 samples can be extracted simultaneously.

This study has shown that the fluctuation test and the plate test perform similarly in the detection of formed genotoxic activity in UV/H_2O_2- treated water. With the advantages of the fluctuation test described above, the fluctuation test is preferred for further analyses of UV/H_2O_2- treated water. This enables the use of commercial SPE cartridges, with the advantages give above. This study has shown that Oasis® HLB performs best among the tested extraction methods and materials. Therefore, extraction with Oasis® HLB is preferred for the extraction of UV/H_2O_2- treated water for the Ames fluctuation test.

In summary, the Ames fluctuation test with Oasis® HLB extraction is preferred for the genotoxicity analysis of UV/H_2O_2- treated water.

4 Conclusions and recommendations

This study has found that Oasis® HLB extracts the genotoxic compounds in MP UV/H_2O_2- treated water best, but XAD extracts these compounds quite well, too. The difference in extraction method can therefore not explain the different outcomes seen by Heringa *et al.* (2009) and Penders *et al.* (IUVA 2009). Additionally, the Ames plate test and the Ames fluctuation test both detected a dose-related response in extracts of the MP UV/H_2O_2- treated water, which was above the threshold of significance at the employed reference doses in both test versions. Based on the TTC, in both test versions health risks cannot be excluded for water producing such a response. The two test versions therefore appear to agree well in the analysis of this MP UV/H_2O_2- treated water, which does not provide an explanation for the different results found by Heringa *et al.* (2011) and Penders *et al.* (IUVA 2009).
No genotoxic responses were detected in the XAD-4 and XAD-2 + XAD-8 extracts of the UV/H_2O_2-treated water in the comet assay. This provides no explanation for the differences found by Heringa *et al.* (2011) and Kashinkunti *et al.* (WQTC 2009).
Lastly, the coconut charcoal extract did not cause any genotoxic response, in contrast to the Oasis® HLB extract. This indicates the observed genotoxicity in Oasis® HLB extracts is not caused by hydrophilic nitrosamines. These results might also be an indication that the genotoxic substances in MP UV/H_2O_2-treated water are not removed by adsorption in GAC, but by another process, e.g. microbial degradation.

Because of the good performance compared to the other methods, and the practical advantages, the Ames fluctuation test with Oasis® HLB extraction is preferred for the genotoxicity analysis of UV/H_2O_2-treated water.

None of the different results found by the different authors could be explained by this comparison study. This comparison study was performed in one laboratory. Possibly, small differences in procedure steps as performed by the different laboratories employed by the different authors, are responsible for the observed differences. It happens regularly that different laboratories find different results, without a clear cause. To verify if the differences come from the employment of different laboratories, a small round robin study could be undertaken to have the different laboratories extract duplicates of the same water, and exchange the duplicate extracts for genotoxicity analysis to make a cross-comparison. Additionally, an exchange of technicians could be made, to enable discovery of small differences in methods.

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I Extraction procedure with Oasis® HLB on manifold

Materials
All chemicals were of analytical grade.
Sodium sulfite (Na_2SO_3) was purchased from J.T Baker (Phillipsburg, NJ, USA.).
Distilled acetone, distilled petroleum ether, ethylacetate, methanol, and acetonitril were purchased from Mallinckrodt Baker B.V. (Deventer, the Netherlands). Hydrochloric acid (Suprapur®, 30%) was obtained from Merck (Darmstadt, Germany). The SPE columns (200 mg Oasis® HLB 5cc LP glass cartridges) came from Waters Corporation (Milford, USA). Filtration columns (empty 8 mL glass column with frit), air cleaning columns (8 mL octadecyl glass column) and sea sand (washed and ignited) were all purchased from Mallinckrodt Baker B.V. (Deventer, The Netherlands).

Sample extraction and concentration
To prevent any contamination during the extraction procedure, only glass, Teflon and stainless steel equipment was used. All materials were extensively washed and then rinsed with distilled acetone and distilled petroleum ether before use, except for Teflon tubes, which were rinsed with ethylacetate.
Before extraction, the samples were brought to pH 2.3 with a 15% ultrapure HCl-solution in Evian mineral water. Glass filtration columns were prepared with sea sand. Filtration and SPE (Oasis® HLB) columns were rinsed twice with full column volumes of 20% methanol in acetonitril, dried and rinsed twice with full column volumes of Evian mineral water brought to pH 2.3. The columns were subsequently filled with fresh Evian mineral water of pH 2.3 and the filtration columns were mounted on the SPE columns. The air cleaning columns were conditioned with one volume of ethylacetate and mounted on the sample bottles.
One litre of a sample was passed through each column setup at around 10 mL/min under low vacuum. Then, the filtration columns were removed and the SPE columns rinsed by 2 column volumes of Evian water of pH 2.3 and dried for one hour. Elution was performed with 3 serial additions of 2.5 mL of 20% methanol in acetonitril (1 min incubation). The 7.5-mL eluates were collected in glass test tubes and stored at -18°C until further processing.
All extracts were evaporated under a gentle stream of nitrogen at 56°C to a volume of 0.5 mL and transferred to a pre-weighted glass conical vial. The test tubes were rinsed with 0.5 mL of acetonitril, which was added to the extract. The acetonitril was further evaporated to approximately 50 µL under a nitrogen stream at 56°C. Then 50 µL of DMSO was added as a keeper and final solvent, and the remaining methanol:acetonitril was evaporated under a nitrogen stream of 65°C in another 10 minutes. Co-evaporated DMSO was replenished to 100 µL by weight, yielding 10,000-fold concentrated extracts. All extracts were stored at -18°C until analysis.

II Procedure Ames fluctuation test

Materials
All chemicals were of analytical grade.
4-Nitroquinoline oxide (4-NQO), 2-aminoanthracene (2-AA) and nitrofurantoin (NF) were purchased from Sigma Aldrich (St. Louis, USA). Dimethylsulfoxide (DMSO) was purchased from Acros Organics (Geel, Belgium). S9-liver enzyme fraction of Spraque-Dawley rats exposed to 1254 Aroclor was purchased from MP Biomedicals Europe (Illkirch, France).

Ames fluctuation test
The Ames fluctuation test strains (TA98, TAMix and /or TA100) and media were purchased from Xenometrix (Basel, Switserland). The test procedure provided by Xenometrix, also described by Fluckiger-Isler et al. (2004), was followed, with minor modifications. Bacterial stock culture was thawed and grown overnight at 37 °C and 250 rpm in a mixture of 10 mL Growth Medium, 10 µL of 50 mg/mL ampicillin-solution and 10 µL stock culture. Growth was checked after 14-17 h by optical density (OD) measurement at 600 nm, and had to be at least 2.0 (or 0.2 for a 10-fold dilution) for continuation of the test. S9-liver enzyme fraction was freshly thawed and mixed as: 33 µL 1 M KCl, 32 µL 0,25 M $MgCl_2 \cdot 6H_2O$, 25 µL 0,2 M Glucose-6-phophate, 100 µL 0,04 M NADP, 500 µL 0,2 M NaH_2PO_4 buffer, 10 µL milliQ water, and 300 µL S9-fraction.[2]
Per well of a 24-well plate (Greiner Bio One), the following was added: 6 µL of diluted test sample in 100% DMSO, 30 µL overnight culture, 10 µL of S9-mix if applicable and 264 or 254 µL of Exposure Medium, respectively. Water extracts were tested in triplicate, as well as a triplicate negative control (DMSO only), a triplicate positive control for genotoxicity (table II-1), and a triplicate positive control for cytotoxicity (1 mg/mL 4-NQO in DMSO). After an incubation of 90 minutes at 37 °C and 250 rpm, 10 µL from each exposure mixture was transferred to a well of a 96-well plate (Greiner Bio One) for a cytotoxicity measurement. To each well of the 96-well plate, 90 µL of Exposure Medium (containing histidine) was added and this was then left to incubate for another 3 hours at 37 °C and 250 rpm. Then, the OD at 595 nm of the 96-well plate was measured with an Opsys MR platereader (Clindia; Leusden, the Netherlands).

Table II-1. Positive controls for the different strains and S9-conditions

Strain and S9-condition	Positive control (in DMSO)
TA98 –S9	20 µg/mL 4-NQO
TA98 +S9	5 µg/mL 2-AA
TAMix –S9	10 µg/mL 4-NQO
TAMix +S9	100 µg/mL 2-AA
TA100 –S9	12.5 µg/mL NF
TA100 + S9	10 µg/mL 2-AA

To the remaining exposure mixture in the 24-well plate, 2.61 mL of purple Indicator Medium (not containing histidine) was added. The total 2.9 mL was subsequently divided over 48 wells (50 µL per well) of a 384 well plate and left to incubate for 48 hours at 37 °C. Then, the number of yellow wells per 48 wells of one sample was counted manually.
As Ames II test responses are not normally distributed, but follow a binomial distribution (Piegorsch et al., 2000), no standard statistical tests could be performed on the data. As an alternative, a water extract was determined to be genotoxic if the number of yellow wells exceeded the detection limit of the test. We

[2] The Ames II assay is performed both with and without S9 liver enzyme extract, in order to detect both direct genotoxic compounds, and indirect genotoxic compounds that need to be converted to a genotoxic metabolite by liver enzymes first.

defined the detection limit (DL) as the value that will only be exceeded by values of the negative control with a very low probability (1%). From statistical theory we may assume that the total number of yellow wells from the three replicates follows a binomial distribution. Therefore we can approach the detection limit of the total number of yellow wells from the three replicates (X) as the smallest integer k that satisfies the following equation (based upon the formula for the cumulative binomial distribution, equation I-1):

$$P[X \le k] = \sum_{i=0}^{k} \binom{n}{i} p^i (1-p)^{n-i} \ge 99\% \qquad \text{equation I-1}$$

With n the total number of wells (n = 144), and p the probability of a yellow well in testing three replicates of a negative control sample. p is estimated as the total number of yellow wells (y) from the three replicates of a negative control sample, divided by the total number of wells involved (144), so p = y/144.

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III Procedure comet assay

Materials

All chemicals were of analytical grade.
Benzo[a]pyrene (BaP), methyl methane sulphonate (MMS), DMSO, neutral red, triton X-100 and ethidium bromide were purchased from Sigma Aldrich (St. Louis, USA). Hank's balanced salt solution (HBSS) was obtained from Invitrogen (Paisley, UK). Dulbecco's modified Eagle's medium (DMEM), Hank's balanced salt solution (HBSS) pencillin/streptomycin and 0.5% trypsin-EDTA solution were was obtained from Invitrogen, Paisley UK. Foetal calf serum (FCS) was obtained from BioWhitaker, Walkersville, USA. Normal melting agarose was obtained from Biozym, Valkenswaard, the Netherlands. Low melting agarose was obtained from Cambrex, Rockland, USA.

Comet assay

The human HepG2 hepatoma cell line was obtained from Dr. B. Knowles of The Wistar Institute of Anatomy and Biology in Philadelphia (Knowles et al., 1980). The cells were grown in a monolayer culture in DMEM supplemented with 10% FCS, 100 U/mL penicillin and 100 µg/mL streptomycin. HepG2 cells were seeded at a density of ± 2×10^6 cells in 75 cm^2 tissue culture flasks and cultured in a humidified incubator (Sanyo, Bensenville, USA) at 37°C and 5% CO_2. Near confluence, HepG2 cells were harvested by trypsination, suspended in culture medium, and split twice a week to prepare subcultures.

For the neutral red assay for cytotoxicity, HepG2 cells were seeded at a density of ca. 5×10^4 cells per well in 96-well culture plates and cultured for two days. The medium was removed and cells were treated with HBSS containing aliquots of the water extracts at a concentration of 0.25%, 0.5% or 1% (v/v) in quadruplicate. Triton X-100 at a concentration of 1% in HBSS (v/v) was used as positive control substance. After a 24-h treatment in a humidified incubator, cells were washed twice with HBSS, followed by exposure to 50 µg/mL neutral red solution in HBSS for 1 h in a humidified incubator. After washing, the incorporated neutral red was then extracted by incubation in 0.02% acetic acid in 50% aqueous ethanol (v/v) for ca. 20 min. Absorbance of the extracted neutral red was measured at 540 nm by means of a spectrophotometer (Biorad, Hercules, USA) and the mean optical density for quadruplicate cultures was calculated and expressed as the percentage of neutral red uptake (viability) compared to the negative control substance DMSO. The water extracts were considered not cytotoxic if viability was greater than 90%, slightly cytotoxic if viability was between 70% and 90%, and cytotoxic if viability was less than 70%. The concentration of the water extracts to be used in the comet assay should demonstrate viability greater than 70%.

For the comet assay, HepG2 cells were seeded at a density of ca. 2.5×10^5 cells per well in 24-well culture plates and cultured for two days. The medium was removed and cells were treated with HBSS containing aliquots of water extract at a concentration of 1% (v/v) in duplicate (exposure to a 200-fold concentration of the water samples). The cells were exposed for 3 h and for 24 h exposure, the positive controls were 25 µg/mL MMS and 50 µg/mL BaP in DMSO, respectively.
After the treatment in a humidified incubator, cells were washed twice with HBSS. Cells were harvested with 0.05 % trypsin-EDTA solution and suspended in 200 µL HBSS to obtain single cells in suspension. Microscopic slides were prepared by mixing 20 µL of the cell suspension with 90 µL 0.5% low-melting agarose solution in PBS. Subsequently, 95 µL of this mixture was loaded on a glass slide, which was precoated with 1.5% normal melting agarose solution, and mounted with a cover slip. The slides were stored on a cold plate until the agarose had coagulated, followed by removal of the cover slip and incubation in lysisbuffer (2.5 M NaCl, 0.1 M Na2EDTA, 0.01 M Tris,1% Triton X-100, pH 10) at ± 4°C for overnight lysis. Slides were then transferred to an electrophoresis box (Biozym, Valkenswaard, The Netherlands) containing ice-cold electrophoresis buffer (0.3 M NaOH, 0.001 M Na2EDTA, pH > 13) and incubated for 30 min to allow DNA unwinding. Electrophoresis was performed for 30 min at 25V and 300 mA at ± 4°C. After electrophoresis, slides were rinsed with neutralization buffer (0.4 M Tris, pH 7.5) and dehydrated with ethanol at room temperature. Slides were stained with 20 µg/mL ethidium

bromide solution, which was directly pipetted on the slide and covered with a cover slip just before analysis. Slides were coded by a qualified person not involved in analyzing the slides to enable blind scoring. A fluorescent microscope (Zeiss, Göttingen, Germany) equipped with a filter (BP 546 nm, FT 580 nm and LP 590 nm) was used for the analysis of the slides. Two slides per culture and fifty randomly selected cells per slide were measured using Comet Assay IV software (Perceptive Instruments, Suffolk, UK). The DNA damage was evaluated by calculation of the mean % tail DNA for a total of two-hundred cells per sample. The water extracts were considered positive when a three fold increase in tail intensity was observed. 'Hedgehog' or 'ghost' cells were excluded from measurement, but their presence was counted to provide and indication of cytotoxicity. Hedgehog cells have the appearance of a small head with a large tail, and have been associated with cells undergoing apoptosis (Meintières et al., 2003).

References

Knowles, B.B., Howe, C.C., Aden, D.P. (1980) Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. Science 209, 497 – 499.

Meintières, S., Nesslany, F., Pallardy, M., Marzin, D. (2003) Detection of ghost cells in the standard alkaline comet assay is not a good measure of apoptosis. Environmental and molecular mutagenesis 41, 260 – 269

IV Raw data of preparative experiment 1

Cytotoxicity data

TA98 -S9		cytotoxicity response (triplicate) OD at 595 nm					statistics cytotoxicity NC < 0,100	NC>0,100			
vial code	sample name	1	2	3	average	stdev	DL	DL	x < DL?	% cytotoxic	cytotoxic?
NC	NC	0,064	0,062	0,056	0,0607	0,0042	0,0569	0,039189		100%	
POS	PC	0,057	0,059	0,057	0,0577	0,0012	0,0569	0,039189		95%	
CYT	PC-cyt	0,044	0,038	0,037	0,0397	0,0038	0,0569	0,039189	Yes	65%	Yes
M093253	Post SF + sulphite	0,06	0,06	0,066	0,0620	0,0035	0,0569	0,039189		102%	
M093254	Post MP UV-ox	0,067	0,062	0,067	0,0653	0,0029	0,0569	0,039189		108%	
M093255	Post MP UV-ox + sulphite	0,063	0,064	0,065	0,0640	0,0010	0,0569	0,039189		105%	
M093256	Post MP UV-ox + catalase	0,068	0,062	0,062	0,0640	0,0035	0,0569	0,039189		105%	
M093257	Post MP UV	0,061	0,064	0,066	0,0637	0,0025	0,0569	0,039189		105%	
M093258	Post MP UV + sulphite	0,068	0,064	0,06	0,0640	0,0040	0,0569	0,039189		105%	
M093259	Post MP UV + catalase	0,062	0,059	0,064	0,0617	0,0025	0,0569	0,039189		102%	
M093260	Post LP UV + sulphite	0,067	0,064	0,063	0,0647	0,0021	0,0569	0,039189		107%	
M093261	Post LP UV-ox + sulphite	0,058	0,063	0,066	0,0623	0,0040	0,0569	0,039189		103%	
M093262	Post MP UV-ox-GAC + sulphite	0,067	0,057	0,055	0,0597	0,0064	0,0569	0,039189		98%	
M093263	Evian	0,062	0,054	0,061	0,0590	0,0044	0,0569	0,039189		97%	
M093264	Evian + sulphite	0,065	0,056	0,065	0,0620	0,0052	0,0569	0,039189		102%	
M093265	Evian + catalase	0,066	0,063	0,063	0,0640	0,0017	0,0569	0,039189		105%	

repeated:

TA98 -S9		cytotoxicity response (triplicate) OD at 595 nm					statistics cytotoxicity NC < 0,100	NC>0,100			
vial code	sample name	1	2	3	average	stdev	DL	DL	x < DL?	% cytotoxic	cytotoxic?
NC	NC	0,071	0,063	0,065	0,0663	0,0042	0,0569	0,044856		109%	
POS	PC	0,068	0,066	0,071	0,0683	0,0025	0,0569	0,044856		113%	
CYT	PC-cyt	0,045	0,04	0,039	0,0413	0,0032	0,0569	0,044856	Yes	68%	Yes
M093253	Post SF + sulphite	0,082	0,077	0,086	0,0817	0,0045	0,0569	0,044856		135%	
M093254	Post MP UV-ox	0,084	0,083	0,089	0,0853	0,0032	0,0569	0,044856		141%	
M093255	Post MP UV-ox + sulphite	0,085	0,087	0,096	0,0893	0,0059	0,0569	0,044856		147%	
M093256	Post MP UV-ox + catalase	0,088	0,09	0,11	0,0960	0,0122	0,0569	0,044856		158%	
M093257	Post MP UV	0,088	0,084	0,095	0,0890	0,0056	0,0569	0,044856		147%	
M093258	Post MP UV + sulphite	0,084	0,084	0,084	0,0840	0,0000	0,0569	0,044856		138%	
M093259	Post MP UV + catalase	0,079	0,093	0,103	0,0917	0,0121	0,0569	0,044856		151%	
M093260	Post LP UV + sulphite	0,077	0,094	0,084	0,0850	0,0085	0,0569	0,044856		140%	
M093261	Post LP UV-ox + sulphite	0,085	0,09	0,093	0,0893	0,0040	0,0569	0,044856		147%	
M093262	Post MP UV-ox-GAC + sulphite	0,086	0,081	0,086	0,0843	0,0029	0,0569	0,044856		139%	
M093263	Evian	0,082	0,09	0,082	0,0847	0,0046	0,0569	0,044856		140%	
M093264	Evian + sulphite	0,08	0,083	0,082	0,0817	0,0015	0,0569	0,044856		135%	
M093265	Evian + catalase	0,083	0,086	0,083	0,0840	0,0017	0,0569	0,044856		138%	

TA98 +S9		cytotoxicity response (triplicate) OD at 595 nm					statistics cytotoxicity			
vial code	sample name	1	2	3	average	stdev	DL	x < DL?	% cytotoxic	cytotoxic?
NC	NC	0,139	0,123	0,112	0,1247	0,0136	0,1181		100%	
POS	PC	0,099	0,084	0,117	0,1000	0,0165	0,1181	Yes	80%	Yes
CYT	PC-cyt	0,048	0,049	0,083	0,0600	0,0199	0,1181	Yes	48%	Yes
M093253	Post SF + sulphite	0,083	0,106	0,1	0,0963	0,0119	0,1181	Yes	77%	Yes
M093254	Post MP UV-ox	0,109	0,09	0,093	0,0973	0,0102	0,1181	Yes	78%	Yes
M093255	Post MP UV-ox + sulphite	0,081	0,089	0,12	0,0967	0,0206	0,1181	Yes	78%	Yes
M093256	Post MP UV-ox + catalase	0,096	0,111	0,115	0,1073	0,0100	0,1181	Yes	86%	Yes
M093257	Post MP UV	0,091	0,103	0,099	0,0977	0,0061	0,1181	Yes	78%	Yes
M093258	Post MP UV + sulphite	0,097	0,07	0,098	0,0883	0,0159	0,1181	Yes	71%	Yes
M093259	Post MP UV + catalase	0,099	0,089	0,092	0,0933	0,0051	0,1181	Yes	75%	Yes
M093260	Post LP UV + sulphite	0,103	0,073	0,087	0,0877	0,0150	0,1181	Yes	70%	Yes
M093261	Post LP UV-ox + sulphite	0,096	0,08	0,091	0,0890	0,0082	0,1181	Yes	71%	Yes
M093262	Post MP UV-ox-GAC + sulphite	0,091	0,111	0,07	0,0907	0,0205	0,1181	Yes	73%	Yes
M093263	Evian	0,084	0,077	0,103	0,0880	0,0135	0,1181	Yes	71%	Yes
M093264	Evian + sulphite	0,108	0,089	0,09	0,0957	0,0107	0,1181	Yes	77%	Yes
M093265	Evian + catalase	0,076	0,08	0,095	0,0837	0,0100	0,1181	Yes	67%	Yes

TAMix -S9		cytotoxicity response (triplicate) OD at 595 nm					statistics cytotoxicity NC < 0,100	NC>0,100			
vial code	sample name	1	2	3	average	stdev	DL	DL	x < DL?	% cytotoxic	cytotoxic?
NC	NC	0,055	0,053	0,052	0,0533	0,0015	0,0500	0,0461		100%	
POS	PC	0,051	0,054	0,053	0,0527	0,0015	0,0500	0,0461		99%	
CYT	PC-cyt	0,038	0,039	0,037	0,0380	0,0010	0,0500	0,0461	Yes	71%	Yes
M093253	Post SF + sulphite	0,056	0,059	0,059	0,0580	0,0017	0,0500	0,0461		109%	
M093254	Post MP UV-ox	0,06	0,059	0,059	0,0593	0,0006	0,0500	0,0461		111%	
M093255	Post MP UV-ox + sulphite	0,056	0,055	0,06	0,0570	0,0026	0,0500	0,0461		107%	
M093256	Post MP UV-ox + catalase	0,061	0,061	0,062	0,0613	0,0006	0,0500	0,0461		115%	
M093257	Post MP UV	0,062	0,067	0,064	0,0643	0,0025	0,0500	0,0461		121%	
M093258	Post MP UV + sulphite	0,058	0,057	0,055	0,0567	0,0015	0,0500	0,0461		106%	
M093259	Post MP UV + catalase	0,056	0,059	0,062	0,0590	0,0030	0,0500	0,0461		111%	
M093260	Post LP UV + sulphite	0,059	0,056	0,057	0,0573	0,0015	0,0500	0,0461		108%	
M093261	Post LP UV-ox + sulphite	0,056	0,062	0,062	0,0600	0,0035	0,0500	0,0461		113%	
M093262	Post MP UV-ox-GAC + sulphite	0,058	0,059	0,055	0,0573	0,0021	0,0500	0,0461		108%	
M093263	Evian	0,056	0,057	0,061	0,0580	0,0026	0,0500	0,0461		109%	
M093264	Evian + sulphite	0,056	0,057	0,056	0,0563	0,0006	0,0500	0,0461		106%	
M093265	Evian + catalase	0,056	0,057	0,061	0,0580	0,0026	0,0500	0,0461		109%	

TAMix +S9		cytotoxicity response (triplicate) OD at 595 nm					statistics cytotoxicity NC < 0,100	NC>0,100			
vial code	sample name	1	2	3	average	stdev	DL	DL	x < DL?	% cytotoxic	cytotoxic?
NC	NC	0,154	0,191	0,16	0,1683	0,0199	0,0965	0,1569		100%	
POS	PC	0,145	0,171	0,151	0,1557	0,0136	0,0965	0,1569	Yes	92%	
CYT	PC-cyt	0,082	0,077	0,079	0,0793	0,0025	0,0965	0,1569	Yes	47%	Yes
M093253	Post SF + sulphite	0,163	0,123	0,163	0,1497	0,0231	0,0965	0,1569	Yes	89%	Yes
M093254	Post MP UV-ox	0,146	0,117	0,136	0,1330	0,0147	0,0965	0,1569	Yes	79%	Yes
M093255	Post MP UV-ox + sulphite	0,142	0,116	0,117	0,1250	0,0147	0,0965	0,1569	Yes	74%	Yes
M093256	Post MP UV-ox + catalase	0,101	0,113	0,112	0,1087	0,0067	0,0965	0,1569	Yes	65%	Yes
M093257	Post MP UV	0,102	0,101	0,107	0,1033	0,0032	0,0965	0,1569	Yes	61%	Yes
M093258	Post MP UV + sulphite	0,125	0,109	0,091	0,1083	0,0170	0,0965	0,1569	Yes	64%	Yes
M093259	Post MP UV + catalase	0,108	0,104	0,104	0,1053	0,0023	0,0965	0,1569	Yes	63%	Yes
M093260	Post LP UV + sulphite	0,121	0,092	0,142	0,1183	0,0251	0,0965	0,1569	Yes	70%	Yes
M093261	Post LP UV-ox + sulphite	0,103	0,107	0,112	0,1073	0,0045	0,0965	0,1569	Yes	64%	Yes
M093262	Post MP UV-ox-GAC + sulphite	0,095	0,128	0,135	0,1193	0,0214	0,0965	0,1569	Yes	71%	Yes
M093263	Evian	0,11	0,069	0,109	0,0960	0,0234	0,0965	0,1569	Yes	57%	Yes
M093264	Evian + sulphite	0,068	0,106	0,084	0,0860	0,0191	0,0965	0,1569	Yes	51%	Yes
M093265	Evian + catalase	0,094	0,097	0,068	0,0863	0,0159	0,0965	0,1569	Yes	51%	Yes

Genotoxicity data:

TA98 -S9		genotoxicity response (triplicate) # yellow wells / 48						statistics genotoxicity	
vial code	sample name	1	2	3	sum	average	stdev	DL	genotoxic? (sum>DL?)
NC	NC	5	3	0	8	2,67	2,52	15	
POS	PC	30	30	33	93	31,00	1,73		Yes
CYT	PC-cyt	0	0	0	0	0,00	0,00		
M093253	Post SF + sulphite	5	6	5	16	5,33	0,58		Yes
M093254	Post MP UV-ox	32	39	33	104	34,67	3,79		Yes
M093255	Post MP UV-ox + sulphite	34	31	32	97	32,33	1,53		Yes
M093256	Post MP UV-ox + catalase	30	28	29	87	29,00	1,00		Yes
M093257	Post MP UV	37	37	37	111	37,00	0,00		Yes
M093258	Post MP UV + sulphite	43	38	27	108	36,00	8,19		Yes
M093259	Post MP UV + catalase	37	39	40	116	38,67	1,53		Yes
M093260	Post LP UV + sulphite	9	6	5	20	6,67	2,08		Yes
M093261	Post LP UV-ox + sulphite	5	6	6	17	5,67	0,58		Yes
M093262	Post MP UV-ox-GAC + sulphite	5	4	8	17	5,67	2,08		Yes
M093263	Evian	7	9	5	21	7,00	2,00		Yes
M093264	Evian + sulphite	2	5	6	13	4,33	2,08		
M093265	Evian + catalase	5	2	8	15	5,00	3,00		

repeated:

TA98 -S9		genotoxicity response (triplicate) # yellow wells / 48						statistics genotoxicity	
vial code	sample name	1	2	3	sum	average	stdev	DL	genotoxic? (sum>DL?)
NC	NC	2	2	1	5	1,67	0,58	11	
POS	PC	25	23	26	74	24,67	1,53		Yes
CYT	PC-cyt	0	0	0	0	0,00	0,00		
M093253	Post SF + sulphite	2	2	1	5	1,67	0,58		
M093254	Post MP UV-ox	31	21	22	74	24,67	5,51		Yes
M093255	Post MP UV-ox + sulphite	20	18	15	53	17,67	2,52		Yes
M093256	Post MP UV-ox + catalase	16	21	16	53	17,67	2,89		Yes
M093257	Post MP UV	32	32	26	90	30,00	3,46		Yes
M093258	Post MP UV + sulphite	26	34	16	76	25,33	9,02		Yes
M093259	Post MP UV + catalase	35	33	34	102	34,00	1,00		Yes
M093260	Post LP UV + sulphite	4	5	3	12	4,00	1,00		Yes
M093261	Post LP UV-ox + sulphite	2	9	2	13	4,33	4,04		Yes
M093262	Post MP UV-ox-GAC + sulphite	1	1	2	4	1,33	0,58		
M093263	Evian	1	2	1	4	1,33	0,58		
M093264	Evian + sulphite	2	3	0	5	1,67	1,53		
M093265	Evian + catalase	1	2	0	3	1,00	1,00		

TA98+S9		genotoxicity response (triplicate) # yellow wells / 48						statistics genotoxicity	
vial code	sample name	1	2	3	sum	average	stdev	DL	genotoxic? (sum>DL?)
NC	NC	6	2	3	11	3,67	2,08	19	
POS	PC	30	32	36	98	32,67	3,06		Yes
CYT	PC-cyt	0	0	0	0	0,00	0,00		
M093253	Post SF + sulphite	4	4	5	13	4,33	0,58		
M093254	Post MP UV-ox	27	24	27	78	26,00	1,73		Yes
M093255	Post MP UV-ox + sulphite	24	24	19	67	22,33	2,89		Yes
M093256	Post MP UV-ox + catalase	16	18	26	60	20,00	5,29		Yes
M093257	Post MP UV	33	37	28	98	32,67	4,51		Yes
M093258	Post MP UV + sulphite	28	33	29	90	30,00	2,65		Yes
M093259	Post MP UV + catalase	41	31	28	100	33,33	6,81		Yes
M093260	Post LP UV + sulphite	8	9	6	23	7,67	1,53		Yes
M093261	Post LP UV-ox + sulphite	5	7	3	15	5,00	2,00		
M093262	Post MP UV-ox-GAC + sulphite	3	5	2	10	3,33	1,53		
M093263	Evian	3	2	4	9	3,00	1,00		
M093264	Evian + sulphite	5	2	3	10	3,33	1,53		
M093265	Evian + catalase	4	2	2	8	2,67	1,15		

TAMix -S9		genotoxicity response (triplicate) # yellow wells / 48						statistics genotoxicity	
vial code	sample name	1	2	3	sum	average	stdev	DL	genotoxic? (sum>DL?)
NC	NC	1	0	0	1	0,33	0,58	4	
POS	PC	12	14	21	47	15,67	4,73		Yes
CYT	PC-cyt	0	0	0	0	0,00	0,00		
M093253	Post SF + sulphite	0	1	0	1	0,33	0,58		
M093254	Post MP UV-ox	3	1	9	13	4,33	4,16		Yes
M093255	Post MP UV-ox + sulphite	1	1	1	3	1,00	0,00		
M093256	Post MP UV-ox + catalase	0	0	1	1	0,33	0,58		
M093257	Post MP UV	1	1	1	3	1,00	0,00		
M093258	Post MP UV + sulphite	2	1	1	4	1,33	0,58		
M093259	Post MP UV + catalase	2	0	1	3	1,00	1,00		
M093260	Post LP UV + sulphite	0	0	0	0	0,00	0,00		
M093261	Post LP UV-ox + sulphite	1	0	0	1	0,33	0,58		
M093262	Post MP UV-ox-GAC + sulphite	0	0	1	1	0,33	0,58		
M093263	Evian	0	1	0	1	0,33	0,58		
M093264	Evian + sulphite	0	1	0	1	0,33	0,58		
M093265	Evian + catalase	1	0	0	1	0,33	0,58		

TAMix +S9		genotoxicity response (triplicate)						statistics genotoxicity	
		# yellow wells / 48							genotoxic?
vial code	sample name	1	2	3	sum	average	stdev	DL	(sum>DL?)
NC	NC	1	0	0	1	0,33	0,58	4	
POS	PC	28	32	36	96	32,00	4,00		Yes
CYT	PC-cyt	0	0	0	0	0,00	0,00		
M093253	Post SF + sulphite	1	0	0	1	0,33	0,58		
M093254	Post MP UV-ox	2	1	1	4	1,33	0,58		
M093255	Post MP UV-ox + sulphite	0	0	1	1	0,33	0,58		
M093256	Post MP UV-ox + catalase	1	0	0	1	0,33	0,58		
M093257	Post MP UV	1	2	2	5	1,67	0,58		Yes
M093258	Post MP UV + sulphite	1	2	0	3	1,00	1,00		
M093259	Post MP UV + catalase	4	0	2	6	2,00	2,00		Yes
M093260	Post LP UV + sulphite	0	0	0	0	0,00	0,00		
M093261	Post LP UV-ox + sulphite	2	0	0	2	0,67	1,15		
M093262	Post MP UV-ox-GAC + sulphite	0	0	0	0	0,00	0,00		
M093263	Evian	0	0	0	0	0,00	0,00		
M093264	Evian + sulphite	0	0	1	1	0,33	0,58		
M093265	Evian + catalase	1	0	0	1	0,33	0,58		

V Raw data of preparative experiment 2

Cytotoxicity data:

TA98 -S9		cytotoxicity response (triplicate)					statistics cytotoxicity				
		OD at 595 nm					NC < 0,100	NC>0,100			
vial code	sample name	1	2	3	average	stdev	DL	DL	x < DL?	% cytotoxic	cytotoxic?
	NC	0,082	0,082	0,081	0,0817	0,0006	0,0779	0,0779		100%	
	PC	0,073	0,070	0,065	0,0693	0,0040	0,0779	0,0779	Yes	85%	Yes
	PC cytotox	0,047	0,041	0,039	0,0423	0,0042	0,0779	0,0779	Yes	52%	Yes
M100355	MD-UVox - HLB glas	0,102	0,108	0,100	0,1033	0,0042	0,0779	0,0779		127%	
M100356	MD-UVox - HLB glas	0,084	0,095	0,101	0,0933	0,0086	0,0779	0,0779		114%	
M100357	MD-UVox - HLB glas	0,097	0,097	0,093	0,0957	0,0023	0,0779	0,0779		117%	
M100364	MD-UVox - HLB glas	0,108	0,109	0,108	0,1083	0,0006	0,0779	0,0779		133%	
M100365	MD-UVox - HLB glas	0,107	0,102	0,093	0,1007	0,0071	0,0779	0,0779		123%	
M100366	procedureblanco HLB glas	0,073	0,076	0,077	0,0753	0,0021	0,0779	0,0779	Yes	92%	
M100358	MD-UVox - HLB plastic	0,087	0,097	0,093	0,0923	0,0050	0,0779	0,0779		113%	
M100359	MD-UVox - HLB plastic	0,088	0,089	0,086	0,0877	0,0015	0,0779	0,0779		107%	
M100360	MD-UVox - HLB plastic	0,083	0,070	0,089	0,0807	0,0097	0,0779	0,0779		99%	
M100367	MD-UVox - HLB plastic	0,105	0,078	0,077	0,0867	0,0159	0,0779	0,0779		106%	
M100368	MD-UVox - HLB plastic	0,078	0,076	0,085	0,0797	0,0047	0,0779	0,0779		98%	
M100369	procedureblanco HLB plastic	0,076	0,069	0,074	0,0730	0,0036	0,0779	0,0779	Yes	89%	Yes
M100352	MD-UVox - MCX plastic	0,083	0,089	0,079	0,0837	0,0050	0,0779	0,0779		102%	
M100353	MD-UVox - MCX plastic	0,080	0,087	0,082	0,0830	0,0036	0,0779	0,0779		102%	
M100354	MD-UVox - MCX plastic	0,081	0,082	0,080	0,0810	0,0010	0,0779	0,0779		99%	
M100361	MD-UVox - MCX plastic	0,083	0,079	0,076	0,0793	0,0035	0,0779	0,0779		97%	
M100362	MD-UVox - MCX plastic	0,084	0,089	0,083	0,0853	0,0032	0,0779	0,0779		104%	
M100363	procedureblanco MCX plastic	0,072	0,078	0,077	0,0757	0,0032	0,0779	0,0779	Yes	93%	

TA98 +S9		cytotoxicity response (triplicate)					statistics cytotoxicity			
		OD at 595 nm								
vial code	sample name	1	2	3	average	stdev	DL	x < DL?	% cytotoxic	cytotoxic?
	NC	0,061	0,06	0,06	0,0603	0,0006	0,0538		100%	
	PC	0,06	0,052	0,056	0,0560	0,0040	0,0538		93%	
	PC cytotox	0,043	0,043	0,041	0,0423	0,0012	0,0538	Yes	70%	Yes
M100355	MD-UVox - HLB glas	0,07	0,073	0,07	0,0710	0,0017	0,0538		118%	
M100356	MD-UVox - HLB glas	0,071	0,075	0,071	0,0723	0,0023	0,0538		120%	
M100357	MD-UVox - HLB glas	0,072	0,077	0,073	0,0740	0,0026	0,0538		123%	
M100364	MD-UVox - HLB glas	0,074	0,071	0,072	0,0723	0,0015	0,0538		120%	
M100365	MD-UVox - HLB glas	0,075	0,075	0,071	0,0737	0,0023	0,0538		122%	
M100366	procedureblanco HLB glas	0,057	0,056	0,061	0,0580	0,0026	0,0538		96%	
M100358	MD-UVox - HLB plastic	0,067	0,067	0,066	0,0667	0,0006	0,0538		110%	
M100359	MD-UVox - HLB plastic	0,064	0,064	0,068	0,0653	0,0023	0,0538		108%	
M100360	MD-UVox - HLB plastic	0,069	0,07	0,075	0,0713	0,0032	0,0538		118%	
M100367	MD-UVox - HLB plastic	0,069	0,08	0,094	0,0810	0,0125	0,0538		134%	
M100368	MD-UVox - HLB plastic	0,067	0,064	0,063	0,0647	0,0021	0,0538		107%	
M100369	procedureblanco HLB plastic	0,058	0,057	0,056	0,0570	0,0010	0,0538		94%	
M100352	MD-UVox - MCX plastic	0,06	0,059	0,067	0,0620	0,0044	0,0538		103%	
M100353	MD-UVox - MCX plastic	0,058	0,069	0,067	0,0647	0,0059	0,0538		107%	
M100354	MD-UVox - MCX plastic	0,062	0,059	0,062	0,0610	0,0017	0,0538		101%	
M100361	MD-UVox - MCX plastic	0,054	0,061	0,061	0,0587	0,0040	0,0538		97%	
M100362	MD-UVox - MCX plastic	0,069	0,061	0,062	0,0640	0,0044	0,0538		106%	
M100363	procedureblanco MCX plastic	0,058	0,058	0,055	0,0570	0,0017	0,0538		94%	

TAMix -S9		cytotoxicity response (triplicate)					statistics cytotoxicity				
		OD at 595 nm					NC < 0,100	NC>0,100			
vial code	sample name	1	2	3	average	stdev	DL	DL	x < DL?	% cytotoxic	cytotoxic?
	NC	0,055	0,055	0,054	0,0547	0,0006	0,0514	0,0481		100%	
	PC	0,051	0,051	0,055	0,0523	0,0023	0,0514	0,0481		96%	
	PC cytotox	0,037	0,038	0,038	0,0377	0,0006	0,0514	0,0481	Yes	69%	Yes
M100355	MD-UVox - HLB glas	0,064	0,073	0,077	0,0713	0,0067	0,0514	0,0481		130%	
M100356	MD-UVox - HLB glas	0,07	0,079	0,064	0,0710	0,0075	0,0514	0,0481		130%	
M100357	MD-UVox - HLB glas	0,067	0,064	0,056	0,0623	0,0057	0,0514	0,0481		114%	
M100364	MD-UVox - HLB glas	0,067	0,069	0,072	0,0693	0,0025	0,0514	0,0481		127%	
M100365	MD-UVox - HLB glas	0,072	0,065	0,060	0,0657	0,0060	0,0514	0,0481		120%	
M100366	procedureblanco HLB glas	0,056	0,060	0,059	0,0583	0,0021	0,0514	0,0481		107%	
M100358	MD-UVox - HLB plastic	0,064	0,059	0,065	0,0627	0,0032	0,0514	0,0481		115%	
M100359	MD-UVox - HLB plastic	0,06	0,059	0,07	0,0630	0,0061	0,0514	0,0481		115%	
M100360	MD-UVox - HLB plastic	0,062	0,062	0,065	0,0630	0,0017	0,0514	0,0481		115%	
M100367	MD-UVox - HLB plastic	0,064	0,062	0,060	0,0620	0,0020	0,0514	0,0481		113%	
M100368	MD-UVox - HLB plastic	0,061	0,057	0,062	0,0600	0,0026	0,0514	0,0481		110%	
M100369	procedureblanco HLB plastic	0,059	0,055	0,052	0,0553	0,0035	0,0514	0,0481		101%	
M100352	MD-UVox - MCX plastic	0,059	0,056	0,055	0,0567	0,0021	0,0514	0,0481		104%	
M100353	MD-UVox - MCX plastic	0,063	0,056	0,057	0,0587	0,0038	0,0514	0,0481		107%	
M100354	MD-UVox - MCX plastic	0,054	0,064	0,059	0,0590	0,0050	0,0514	0,0481		108%	
M100361	MD-UVox - MCX plastic	0,057	0,062	0,062	0,0603	0,0029	0,0514	0,0481		110%	
M100362	MD-UVox - MCX plastic	0,06	0,063	0,059	0,0607	0,0021	0,0514	0,0481		111%	
M100363	procedureblanco MCX plastic	0,057	0,060	0,057	0,0580	0,0017	0,0514	0,0481		106%	

TAMix +S9		cytotoxicity response (triplicate)					statistics cytotoxicity				
		OD at 595 nm					NC < 0,100	NC>0,100			
vial code	sample name	1	2	3	average	stdev	DL	DL	x < DL?	% cytotoxic	cytotoxic?
	NC	0,062	0,065	0,057	0,0613	0,0040	0,0568	0,0499		100%	
	PC	0,056	0,055	0,055	0,0553	0,0006	0,0568	0,0499	Yes	90%	
	PC cytotox	0,047	0,043	0,047	0,0457	0,0023	0,0568	0,0499	Yes	74%	Yes
M100355	MD-UVox - HLB glas	0,076	0,066	0,063	0,0683	0,0068	0,0568	0,0499		111%	
M100356	MD-UVox - HLB glas	0,064	0,068	0,061	0,0643	0,0035	0,0568	0,0499		105%	
M100357	MD-UVox - HLB glas	0,069	0,065	0,064	0,0660	0,0026	0,0568	0,0499		108%	
M100364	MD-UVox - HLB glas	0,065	0,078	0,064	0,0690	0,0078	0,0568	0,0499		113%	
M100365	MD-UVox - HLB glas	0,069	0,065	0,066	0,0667	0,0021	0,0568	0,0499		109%	
M100366	procedureblanco HLB glas	0,062	0,059	0,067	0,0627	0,0040	0,0568	0,0499		102%	
M100358	MD-UVox - HLB plastic	0,065	0,066	0,062	0,0643	0,0021	0,0568	0,0499		105%	
M100359	MD-UVox - HLB plastic	0,062	0,061	0,062	0,0617	0,0006	0,0568	0,0499		101%	
M100360	MD-UVox - HLB plastic	0,064	0,067	0,062	0,0643	0,0025	0,0568	0,0499		105%	
M100367	MD-UVox - HLB plastic	0,063	0,065	0,062	0,0633	0,0015	0,0568	0,0499		103%	
M100368	MD-UVox - HLB plastic	0,062	0,061	0,062	0,0617	0,0006	0,0568	0,0499		101%	
M100369	procedureblanco HLB plastic	0,060	0,058	0,057	0,0583	0,0015	0,0568	0,0499		95%	
M100352	MD-UVox - MCX plastic	0,058	0,057	0,058	0,0577	0,0006	0,0568	0,0499		94%	
M100353	MD-UVox - MCX plastic	0,068	0,055	0,056	0,0597	0,0072	0,0568	0,0499		97%	
M100354	MD-UVox - MCX plastic	0,060	0,060	0,056	0,0587	0,0023	0,0568	0,0499		96%	
M100361	MD-UVox - MCX plastic	0,061	0,056	0,053	0,0567	0,0040	0,0568	0,0499	Yes	92%	
M100362	MD-UVox - MCX plastic	0,056	0,059	0,059	0,0580	0,0017	0,0568	0,0499		95%	
M100363	procedureblanco MCX plastic	0,065	0,060	0,055	0,0600	0,0050	0,0568	0,0499		98%	

Genotoxicity data:

TA98 -S9		genotoxicity response (triplicate)						statistics genotoxicity	
		# yellow wells / 48							genotoxic?
vial code	sample name	1	2	3	sum	average	stdev	DL	(sum>DL?)
	NC	0	1	1	2	0,67	0,58	6	
	PC	23	27	22	72	24,00	2,65		Yes
	PC cytotox	0	0	0	0	0,00	0,00		
M100355	MD-UVox - HLB glass	41	32	34	107	35,67	4,73		Yes
M100356	MD-UVox - HLB glass	32	36	28	96	32,00	4,00		Yes
M100357	MD-UVox - HLB glass	34	35	28	97	32,33	3,79		Yes
M100364	MD-UVox - HLB glass	40	36	30	106	35,33	5,03		Yes
M100365	MD-UVox - HLB glass	39	40	37	116	38,67	1,53		Yes
M100366	procedurecontrol HLB glass	0	1	2	3	1,00	1,00		
M100358	MD-UVox - HLB plastic	34	36	37	107	35,67	1,53		Yes
M100359	MD-UVox - HLB plastic	36	34	35	105	35,00	1,00		Yes
M100360	MD-UVox - HLB plastic	38	35	32	105	35,00	3,00		Yes
M100367	MD-UVox - HLB plastic	35	36	35	106	35,33	0,58		Yes
M100368	MD-UVox - HLB plastic	35	38	36	109	36,33	1,53		Yes
M100369	procedurecontrol HLB plastic	1	1	3	5	1,67	1,15		
M100352	MD-UVox - MCX plastic	34	32	33	99	33,00	1,00		Yes
M100353	MD-UVox - MCX plastic	39	38	36	113	37,67	1,53		Yes
M100354	MD-UVox - MCX plastic	37	28	34	99	33,00	4,58		Yes
M100361	MD-UVox - MCX plastic	36	34	30	100	33,33	3,06		Yes
M100362	MD-UVox - MCX plastic	39	28	31	98	32,67	5,69		Yes
M100363	procedurecontrol MCX plastic	1	2	1	4	1,33	0,58		

TA98 +S9		genotoxicity response (triplicate)						statistics genotoxicity	
		# yellow wells / 48							genotoxic?
vial code	sample name	1	2	3	sum	average	stdev	DL	(sum>DL?)
	NC	1	3	2	6	2,00	1,00	12	
	PC	26	27	22	75	25,00	2,65		Yes
	PC cytotox	0	0	0	0	0,00	0,00		
M100355	MD-UVox - HLB glass	28	32	28	88	29,33	2,31		Yes
M100356	MD-UVox - HLB glass	34	39	36	109	36,33	2,52		Yes
M100357	MD-UVox - HLB glass	27	25	35	87	29,00	5,29		Yes
M100364	MD-UVox - HLB glass	32	29	29	90	30,00	1,73		Yes
M100365	MD-UVox - HLB glass	31	30	37	98	32,67	3,79		Yes
M100366	procedurecontrol HLB glass	2	4	6	12	4,00	2,00		
M100358	MD-UVox - HLB plastic	33	28	26	87	29,00	3,61		Yes
M100359	MD-UVox - HLB plastic	32	27	26	85	28,33	3,21		Yes
M100360	MD-UVox - HLB plastic	35	33	28	96	32,00	3,61		Yes
M100367	MD-UVox - HLB plastic	33	35	34	102	34,00	1,00		Yes
M100368	MD-UVox - HLB plastic	30	32	36	98	32,67	3,06		Yes
M100369	procedurecontrol HLB plastic	6	6	6	18	6,00	0,00		Yes
M100352	MD-UVox - MCX plastic	25	23	19	67	22,33	3,06		Yes
M100353	MD-UVox - MCX plastic	24	21	18	63	21,00	3,00		Yes
M100354	MD-UVox - MCX plastic	23	23	15	61	20,33	4,62		Yes
M100361	MD-UVox - MCX plastic	22	22	20	64	21,33	1,15		Yes
M100362	MD-UVox - MCX plastic	24	23	14	61	20,33	5,51		Yes
M100363	procedurecontrol MCX plastic	6	6	6	18	6,00	0,00		Yes

TAMix -S9		genotoxicity response (triplicate)						statistics genotoxicity	
		# yellow wells / 48							genotoxic?
vial code	sample name	1	2	3	sum	average	stdev	DL	(sum>DL?)
	NC	0	1	0	1	0,33	0,58	4	
	PC	29	33	25	87	29,00	4,00		Yes
	PC cytotox	0	0	0	0	0,00	0,00		
M100355	MD-UVox - HLB glas	0	0	2	2	0,67	1,15		
M100356	MD-UVox - HLB glas	2	0	1	3	1,00	1,00		
M100357	MD-UVox - HLB glas	2	1	2	5	1,67	0,58		Yes
M100364	MD-UVox - HLB glas	0	0	0	0	0,00	0,00		
M100365	MD-UVox - HLB glas	1	4	1	6	2,00	1,73		Yes
M100366	procedureblanco HLB glas	0	0	1	1	0,33	0,58		
M100358	MD-UVox - HLB plastic	3	0	0	3	1,00	1,73		
M100359	MD-UVox - HLB plastic	1	2	2	5	1,67	0,58		Yes
M100360	MD-UVox - HLB plastic	3	0	1	4	1,33	1,53		
M100367	MD-UVox - HLB plastic	1	1	0	2	0,67	0,58		
M100368	MD-UVox - HLB plastic	0	0	2	2	0,67	1,15		
M100369	procedureblanco HLB plastic	0	0	0	0	0,00	0,00		
M100352	MD-UVox - MCX plastic	2	0	2	4	1,33	1,15		
M100353	MD-UVox - MCX plastic	0	3	1	4	1,33	1,53		
M100354	MD-UVox - MCX plastic	1	3	0	4	1,33	1,53		
M100361	MD-UVox - MCX plastic	1	1	0	2	0,67	0,58		
M100362	MD-UVox - MCX plastic	0	1	0	1	0,33	0,58		
M100363	procedureblanco MCX plastic	0	0	1	1	0,33	0,58		

TAMix +S9		genotoxicity response (triplicate)						statistics genotoxicity	
		# yellow wells / 48							genotoxic?
vial code	sample name	1	2	3	sum	average	stdev	DL	(sum>DL?)
	NC	0	0	1	1	0,33	0,58	4	
	PC	26	28	32	86	28,67	3,06		Yes
	PC cytotox	0	0	0	0	0,00	0,00		
M100355	MD-UVox - HLB glas	0	2	2	4	1,33	1,15		
M100356	MD-UVox - HLB glas	0	3	0	3	1,00	1,73		
M100357	MD-UVox - HLB glas	2	1	1	4	1,33	0,58		
M100364	MD-UVox - HLB glas	1	1	0	2	0,67	0,58		
M100365	MD-UVox - HLB glas	1	2	0	3	1,00	1,00		
M100366	procedureblanco HLB glas	0	0	0	0	0,00	0,00		
M100358	MD-UVox - HLB plastic	0	0	1	1	0,33	0,58		
M100359	MD-UVox - HLB plastic	4	2	0	6	2,00	2,00		Yes
M100360	MD-UVox - HLB plastic	2	4	1	7	2,33	1,53		Yes
M100367	MD-UVox - HLB plastic	2	3	1	6	2,00	1,00		Yes
M100368	MD-UVox - HLB plastic	1	2	0	3	1,00	1,00		
M100369	procedureblanco HLB plastic	2	0	0	2	0,67	1,15		
M100352	MD-UVox - MCX plastic	0	0	1	1	0,33	0,58		
M100353	MD-UVox - MCX plastic	3	0	1	4	1,33	1,53		
M100354	MD-UVox - MCX plastic	1	1	0	2	0,67	0,58		
M100361	MD-UVox - MCX plastic	0	1	1	2	0,67	0,58		
M100362	MD-UVox - MCX plastic	0	0	0	0	0,00	0,00		
M100363	procedureblanco MCX plastic	0	1	0	1	0,33	0,58		

VI Raw data of Ames plate test in comparison study

TA98 -S9			genotoxicity response					evaluation genotoxicity			
			number of revertants							genotoxic?	cytotoxicity
vial code	sample name	dose (uL)	plate 1	plate 2	plate 3	average	stdev	induced rev	ind factor	x>3*NC?	observed?
NC	NC		23	16	19	19,3	3,5				
PC	PC		204	216	205	208,3	6,7	189,0	9,8	Yes	No
M-104827	PrC XAD pH neutral	80	18	13		15,5	3,5	-3,8	-0,2		No
M-104828	PrC XAD pH 2	80	19	16		17,5	2,1	-1,8	-0,1		No
M-104829	PrC Oasis HLB	80	8	14		11,0	4,2	-8,3	-0,4		No
M-104824	MP UVox XAD pH neutral	10	150	152		151,0	1,4	131,7	6,8	Yes	No
	MP UVox XAD pH neutral	20	298	331		314,5	23,3	295,2	15,3	Yes	No
	MP UVox XAD pH neutral	40	573	536		554,5	26,2	535,2	27,7	Yes	No
	MP UVox XAD pH neutral	60	821	861		841,0	28,3	821,7	42,5	Yes	No
	MP UVox XAD pH neutral	80	1036	1089		1062,5	37,5	1043,2	54,0	Yes	No
M-104825	MP UVox XAD pH 2	10	139	103		121,0	25,5	101,7	5,3	Yes	No
	MP UVox XAD pH 2	20	177	179		178,0	1,4	158,7	8,2	Yes	No
	MP UVox XAD pH 2	40	350	354		352,0	2,8	332,7	17,2	Yes	No
	MP UVox XAD pH 2	60	510	484		497,0	18,4	477,7	24,7	Yes	No
	MP UVox XAD pH 2	80	70	674		674,0	#DIV/0!	654,7	33,9	Yes	No
M-104826	MP UVox Oasis HLB	10	244	244		244,0	0,0	224,7	11,6	Yes	No
	MP UVox Oasis HLB	20	380	369		374,5	7,8	355,2	18,4	Yes	No
	MP UVox Oasis HLB	40	600	634		617,0	24,0	597,7	30,9	Yes	No
	MP UVox Oasis HLB	60	875	970		922,5	67,2	903,2	46,7	Yes	No
	MP UVox Oasis HLB	80	1189	1081		1135,0	76,4	1115,7	57,7	Yes	No

strange response, left out of calculations

TA98 +S9			genotoxicity response					evaluation genotoxicity			
			number of revertants							genotoxic?	cytotoxicity
vial code	sample name	dose (uL)	plate 1	plate 2	plate 3	average	stdev	induced rev	ind factor	x>3*NC?	observed?
NC	NC		24	22	18	21,3	3,1				
PC	PC		111	107	98	105,3	6,7	84,0	3,9	Yes	No
M-104827	PrC XAD pH neutral	80	22	29		25,5	4,9	4,2	0,2		No
M-104828	PrC XAD pH 2	80	29	23		26,0	4,2	4,7	0,2		No
M-104829	PrC Oasis HLB	80	23	26		24,5	2,1	3,2	0,1		No
M-104824	MP UVox XAD pH neutral	10	92	99		95,5	4,9	74,2	3,5	Yes	No
	MP UVox XAD pH neutral	20	157	179		168,0	15,6	146,7	6,9	Yes	No
	MP UVox XAD pH neutral	40	398	300		349,0	69,3	327,7	15,4	Yes	No
	MP UVox XAD pH neutral	60	509	515		512,0	4,2	490,7	23,0	Yes	No
	MP UVox XAD pH neutral	80	621	631		626,0	7,1	604,7	28,3	Yes	No
M-104825	MP UVox XAD pH 2	10	73	76		74,5	2,1	53,2	2,5	Yes	No
	MP UVox XAD pH 2	20	139	184		161,5	31,8	140,2	6,6	Yes	No
	MP UVox XAD pH 2	40	291	321		306,0	21,2	284,7	13,3	Yes	No
	MP UVox XAD pH 2	60	509	459		484,0	35,4	462,7	21,7	Yes	No
	MP UVox XAD pH 2	80	642	649		645,5	4,9	624,2	29,3	Yes	No
M-104826	MP UVox Oasis HLB	10	163	170		166,5	4,9	145,2	6,8	Yes	No
	MP UVox Oasis HLB	20	337	330		333,5	4,9	312,2	14,6	Yes	No
	MP UVox Oasis HLB	40	665	652		658,5	9,2	637,2	29,9	Yes	No
	MP UVox Oasis HLB	60	1028	1010		1019,0	12,7	997,7	46,8	Yes	No
	MP UVox Oasis HLB	80	1178	1172		1175,0	4,2	1153,7	54,1	Yes	No

TA100 -S9			genotoxicity response					evaluation genotoxicity			
			number of revertants							genotoxic?	cytotoxicity
vial code	sample name	dose (uL)	plate 1	plate 2	plate 3	average	stdev	induced rev	ind factor	x>3*NC?	observed?
NC	NC		131	135	131	132,3	2,3				
PC	PC		378	364	365	369,0	7,8	236,7	1,8		No
M-104827	PrC XAD pH neutral	80	124	127		125,5	2,1	-6,8	-0,1		No
M-104828	PrC XAD pH 2	80	109	106		107,5	2,1	-24,8	-0,2		No
M-104829	PrC Oasis HLB	80	151	125		138,0	18,4	5,7	0,0		No
M-104824	MP UVox XAD pH neutral	10	131	125		128,0	4,2	-4,3	0,0		No
	MP UVox XAD pH neutral	20	168	156		162,0	8,5	29,7	0,2		No
	MP UVox XAD pH neutral	40	174	250		212,0	53,7	79,7	0,6		No
	MP UVox XAD pH neutral	60	251	291		271,0	28,3	138,7	1,0		No
	MP UVox XAD pH neutral	80	334	276		305,0	41,0	172,7	1,3		No
M-104825	MP UVox XAD pH 2	10	131	151		141,0	14,1	8,7	0,1		No
	MP UVox XAD pH 2	20	155	136		145,5	13,4	13,2	0,1		No
	MP UVox XAD pH 2	40	223	196		209,5	19,1	77,2	0,6		No
	MP UVox XAD pH 2	60	222	227		224,5	3,5	92,2	0,7		No
	MP UVox XAD pH 2	80	317	285		301,0	22,6	168,7	1,3		No
M-104826	MP UVox Oasis HLB	10	160	173		166,5	9,2	34,2	0,3		No
	MP UVox Oasis HLB	20	194	219		206,5	17,7	74,2	0,6		No
	MP UVox Oasis HLB	40	270	253		261,5	12,0	129,2	1,0		No
	MP UVox Oasis HLB	60	331	328		329,5	2,1	197,2	1,5		No
	MP UVox Oasis HLB	80	391	409		400,0	12,7	267,7	2,0	Yes	No

TA100 +S9			genotoxicity response					evaluation genotoxicity			
			number of revertants							genotoxic?	cytotoxicity
vial code	sample name	dose (uL)	plate 1	plate 2	plate 3	average	stdev	induced rev	ind factor	x>3*NC?	observed?
NC	NC		157	115	130	134,0	21,3				
PC	PC		204	156	174	178,0	24,2	44,0	0,3		No
M-104827	PrC XAD pH neutral	80	145	122		133,5	16,3	1,2	0,0		No
M-104828	PrC XAD pH 2	80	136	120		128,0	11,3	-4,3	0,0		No
M-104829	PrC Oasis HLB	80	164	145		154,5	13,4	22,2	0,2		No
M-104824	MP UVox XAD pH neutral	10	137	131		134,0	4,2	1,7	0,0		No
	MP UVox XAD pH neutral	20	159	165		162,0	4,2	29,7	0,2		No
	MP UVox XAD pH neutral	40	208	145		176,5	44,5	44,2	0,3		No
	MP UVox XAD pH neutral	60	185	215		200,0	21,2	67,7	0,5		No
	MP UVox XAD pH neutral	80	209	214		211,5	3,5	79,2	0,6		No
M-104825	MP UVox XAD pH 2	10	183	155		169,0	19,8	36,7	0,3		No
	MP UVox XAD pH 2	20	149	149		149,0	0,0	16,7	0,1		No
	MP UVox XAD pH 2	40	199	192		195,5	4,9	63,2	0,5		No
	MP UVox XAD pH 2	60	207	226		216,5	13,4	84,2	0,6		No
	MP UVox XAD pH 2	80	227	299		263,0	50,9	130,7	1,0		No
M-104826	MP UVox Oasis HLB	10	177	174		175,5	2,1	43,2	0,3		No
	MP UVox Oasis HLB	20	198	177		187,5	14,8	55,2	0,4		No
	MP UVox Oasis HLB	40	214	208		211,0	4,2	78,7	0,6		No
	MP UVox Oasis HLB	60	281	320		300,5	27,6	168,2	1,3		No
	MP UVox Oasis HLB	80	334	428		381,0	66,5	248,7	1,9		No

VII Raw data of Ames fluctuation test in comparison study

Cytotoxicity data:

TA98 -S9			cytotoxicity response					statistics cytotoxicity				
			OD at 595 nm					NC < 0,100	NC>0,100			
vial code	sample name	dose (uL)	well 1	well 2	well 3	average	stdev	DL	DL	x < DL?	% cytotoxic	cytotoxic?
	NC (DMSO)	6	0,087	0,084	0,065	0,0787	0,0119	0,0749	0,0572		100%	
	PC	6	0,067	0,100	0,089	0,0853	0,0168	0,0749	0,0572		108%	
	PC cytotox	6	0,043	0,063	0,057	0,0543	0,0103	0,0749	0,0572	Yes	69%	Yes
08-10-2010/**25**	PrC Oasis HLB (DMSO)	6	0,072	0,073	0,066	0,0703	0,0038	0,0749	0,0572	Yes	89%	Yes
08-10-2010/**7**	MP UVox Oasis HLB (DMSO)	6	0,074	0,075		0,0745	0,0007	0,0749	0,0572	Yes	95%	
	MP UVox Oasis HLB (DMSO)	5	0,086	0,078		0,0820	0,0057	0,0749	0,0572		104%	
	MP UVox Oasis HLB (DMSO)	4	0,080	0,077		0,0785	0,0021	0,0749	0,0572		100%	
	MP UVox Oasis HLB (DMSO)	3	0,068	0,072		0,0700	0,0028	0,0749	0,0572	Yes	89%	Yes
	MP UVox Oasis HLB (DMSO)	2	0,069	0,072		0,0705	0,0021	0,0749	0,0572	Yes	90%	
	NC (EtOH)	6	0,059	0,059	0,058	0,0587	0,0006	0,0549	0,0372		100%	
08-10-2010/**3**	PrC XAD pH neutral	6	0,078	0,072	0,062	0,0707	0,0081	0,0549	0,0372		120%	
08-10-2010/**5**	PrC XAD pH 2	6	0,066	0,075	0,072	0,0710	0,0046	0,0549	0,0372		121%	
08-10-2010/**27**	PrC Oasis HLB	6	0,068	0,067	0,067	0,0673	0,0006	0,0549	0,0372		115%	
08-10-2010/**43**	PrC coconut charcoal	6	0,063	0,066	0,067	0,0653	0,0021	0,0549	0,0372		111%	
03-09-2010/**98**	MP UVox XAD pH neutral	6	0,066	0,063		0,0645	0,0021	0,0549	0,0372		110%	
	MP UVox XAD pH neutral	5	0,063	0,062		0,0625	0,0007	0,0549	0,0372		107%	
	MP UVox XAD pH neutral	4	0,064	0,063		0,0635	0,0007	0,0549	0,0372		108%	
	MP UVox XAD pH neutral	3	0,062	0,063		0,0625	0,0007	0,0549	0,0372		107%	
	MP UVox XAD pH neutral	2	0,063	0,061		0,0620	0,0014	0,0549	0,0372		106%	
08-10-2010/**1**	MP UVox XAD pH 2	6	0,058	0,063		0,0605	0,0035	0,0549	0,0372		103%	
	MP UVox XAD pH 2	5	0,066	0,063		0,0645	0,0021	0,0549	0,0372		110%	
	MP UVox XAD pH 2	4	0,058	0,064		0,0610	0,0042	0,0549	0,0372		104%	
	MP UVox XAD pH 2	3	0,061	0,066		0,0635	0,0035	0,0549	0,0372		108%	
	MP UVox XAD pH 2	2	0,073	0,071		0,0720	0,0014	0,0549	0,0372		123%	
08-10-2010/**9**	MP UVox Oasis HLB (EtOH)	6	0,075	0,073		0,0740	0,0014	0,0549	0,0372		126%	
	MP UVox Oasis HLB (EtOH)	5	0,067	0,067		0,0670	0,0000	0,0549	0,0372		114%	
	MP UVox Oasis HLB (EtOH)	4	0,066	0,067		0,0665	0,0007	0,0549	0,0372		113%	
	MP UVox Oasis HLB (EtOH)	3	0,070	0,065		0,0675	0,0035	0,0549	0,0372		115%	
	MP UVox Oasis HLB (EtOH)	2	0,065	0,061		0,0630	0,0028	0,0549	0,0372		107%	
08-10-2010/**42**	MP UVox coconut charcoal (EtOH)	6	0,062	0,067		0,0645	0,0035	0,0549	0,0372		110%	
	MP UVox coconut charcoal (EtOH)	5	0,066	0,062		0,0640	0,0028	0,0549	0,0372		109%	
	MP UVox coconut charcoal (EtOH)	4	0,058	0,060		0,0590	0,0014	0,0549	0,0372		101%	
	MP UVox coconut charcoal (EtOH)	3	0,059	0,066		0,0625	0,0049	0,0549	0,0372		107%	
	MP UVox coconut charcoal (EtOH)	2	0,060	0,060		0,0600	0,0000	0,0549	0,0372		102%	

TA98 +S9			cytotoxicity response					statistics cytotoxicity			
			OD at 595 nm								
vial code	sample name	dose (uL)	well 1	well 2	well 3	gem	stdev	DL	x < DL?	% cytotoxic	cytotoxic?
	NC (DMSO)	6	0,087	0,120	0,099	0,1020	0,0167	0,0954		100%	
	PC	6	0,181	0,106	0,137	0,1413	0,0377	0,0954		139%	
	PC cytotox	6	0,053	0,060	0,074	0,0623	0,0107	0,0954	Yes	61%	Yes
	PrC Oasis HLB (DMSO)	6	0,094	0,111	0,117	0,1073	0,0119	0,0954		105%	
	MP UVox Oasis HLB (DMSO)	6	0,140	0,131		0,1355	0,0064	0,0954		133%	
	MP UVox Oasis HLB (DMSO)	5	0,064	0,111		0,0875	0,0332	0,0954	Yes	86%	Yes
	MP UVox Oasis HLB (DMSO)	4	0,122	0,099		0,1105	0,0163	0,0954		108%	
	MP UVox Oasis HLB (DMSO)	3	0,131	0,122		0,1265	0,0064	0,0954		124%	
	MP UVox Oasis HLB (DMSO)	2	0,129	0,070		0,0995	0,0417	0,0954		98%	
	NC (EtOH)	6	0,088	0,066	0,082	0,0787	0,0114	0,0721		100%	
	PrC XAD pH neutral	6	0,141	0,080	0,125	0,1153	0,0316	0,0721		147%	
	PrC XAD pH 2	6	0,076	0,097	0,094	0,0890	0,0114	0,0721		113%	
	PrC Oasis HLB	6	0,126	0,109	0,086	0,1070	0,0201	0,0721		136%	
	PrC coconut charcoal	6	0,084	0,097	0,082	0,0877	0,0081	0,0721		111%	
	MP UVox XAD pH neutral	6	0,142	0,120		0,1310	0,0156	0,0721		167%	
	MP UVox XAD pH neutral	5	0,098	0,139		0,1185	0,0290	0,0721		151%	
	MP UVox XAD pH neutral	4	0,112	0,087		0,0995	0,0177	0,0721		126%	
	MP UVox XAD pH neutral	3	0,078	0,093		0,0855	0,0106	0,0721		109%	
	MP UVox XAD pH neutral	2	0,084	0,084		0,0840	0,0000	0,0721		107%	
	MP UVox XAD pH 2	6	0,084	0,089		0,0865	0,0035	0,0721		110%	
	MP UVox XAD pH 2	5	0,108	0,095		0,1015	0,0092	0,0721		129%	
	MP UVox XAD pH 2	4	0,093	0,091		0,0920	0,0014	0,0721		117%	
	MP UVox XAD pH 2	3	0,116	0,078		0,0970	0,0269	0,0721		123%	
	MP UVox XAD pH 2	2	0,132	0,114		0,1230	0,0127	0,0721		156%	
	MP UVox Oasis HLB (EtOH)	6	0,144	0,112		0,1280	0,0226	0,0721		163%	
	MP UVox Oasis HLB (EtOH)	5	0,113	0,116		0,1145	0,0021	0,0721		146%	
	MP UVox Oasis HLB (EtOH)	4	0,104	0,111		0,1075	0,0049	0,0721		137%	
	MP UVox Oasis HLB (EtOH)	3	0,103	0,106		0,1045	0,0021	0,0721		133%	
	MP UVox Oasis HLB (EtOH)	2	0,096	0,104		0,1000	0,0057	0,0721		127%	
	MP UVox coconut charcoal (EtOH)	6	0,089	0,089		0,0890	0,0000	0,0721		113%	
	MP UVox coconut charcoal (EtOH)	5	0,175	0,111		0,1430	0,0453	0,0721		182%	
	MP UVox coconut charcoal (EtOH)	4	0,134	0,088		0,1110	0,0325	0,0721		141%	
	MP UVox coconut charcoal (EtOH)	3	0,082	0,138		0,1100	0,0396	0,0721		140%	
	MP UVox coconut charcoal (EtOH)	2	0,146	0,068		0,1070	0,0552	0,0721		136%	

TA100 -S9			cytotoxicity response					statistics cytotoxicity				
			OD at 595 nm					NC < 0,100	NC>0,100			
vial code	sample name	dose (uL)	well 1	well 2	well 3	average	stdev	DL	DL	x < DL?	% cytotoxic	cytotoxic?
	NC (DMSO)	6	0,055	0,059	0,061	0,0583	0,0031	0,0545			100%	
	PC	6	0,057	0,061	0,058	0,0587	0,0021	0,0545			101%	
	PC cytotox	6	0,037	0,037	0,036	0,0367	0,0006	0,0545		Yes	63%	Yes
	PrC Oasis HLB	6	0,056	0,052	0,058	0,0553	0,0031	0,0545			95%	
	MP UVox Oasis HLB (DMSO)	6	0,065			0,0650	#DIV/0!	0,0545			111%	
	MP UVox Oasis HLB (DMSO)	5	0,061	0,066		0,0635	0,0035	0,0545			109%	
	MP UVox Oasis HLB (DMSO)	4	0,059	0,058		0,0585	0,0007	0,0545			100%	
	MP UVox Oasis HLB (DMSO)	3	0,060	0,060		0,0600	0,0000	0,0545			103%	
	MP UVox Oasis HLB (DMSO)	2	0,060	0,063		0,0615	0,0021	0,0545			105%	
	NC (EtOH)	6	0,052	0,049	0,051	0,0507	0,0015	0,0469			100%	
	PrC XAD pH neutral	6	0,053	0,057	0,059	0,0563	0,0031	0,0469			111%	
	PrC XAD pH 2	6	0,060	0,057	0,056	0,0577	0,0021	0,0469			114%	
	PrC Oasis HLB	6	0,060	0,065	0,056	0,0603	0,0045	0,0469			119%	
	PrC coconut charcoal	6	0,061	0,059	0,063	0,0610	0,0020	0,0469			120%	
	MP UVox XAD pH neutral	6	0,066	0,055		0,0605	0,0078	0,0469			119%	
	MP UVox XAD pH neutral	5	0,055	0,058		0,0565	0,0021	0,0469			112%	
	MP UVox XAD pH neutral	4	0,050	0,055		0,0525	0,0035	0,0469			104%	
	MP UVox XAD pH neutral	3	0,056	0,052		0,0540	0,0028	0,0469			107%	
	MP UVox XAD pH neutral	2	0,050	0,051		0,0505	0,0007	0,0469			100%	
	MP UVox XAD pH 2	6	0,060	0,052		0,0560	0,0057	0,0469			111%	
	MP UVox XAD pH 2	5	0,057	0,054		0,0555	0,0021	0,0469			110%	
	MP UVox XAD pH 2	4	0,053	0,054		0,0535	0,0007	0,0469			106%	
	MP UVox XAD pH 2	3	0,055	0,054		0,0545	0,0007	0,0469			108%	
	MP UVox XAD pH 2	2	0,061	0,062		0,0615	0,0007	0,0469			121%	
	MP UVox Oasis HLB (EtOH)	6	0,063	0,061		0,0620	0,0014	0,0469			122%	
	MP UVox Oasis HLB (EtOH)	5	0,055	0,055		0,0550	0,0000	0,0469			109%	
	MP UVox Oasis HLB (EtOH)	4	0,053	0,058		0,0555	0,0035	0,0469			110%	
	MP UVox Oasis HLB (EtOH)	3	0,062	0,063		0,0625	0,0007	0,0469			123%	
	MP UVox Oasis HLB (EtOH)	2	0,056			0,0560	#DIV/0!	0,0469			111%	
	MP UVox coconut charcoal (EtOH)	6	0,059	0,063		0,0610	0,0028	0,0469			120%	
	MP UVox coconut charcoal (EtOH)	5	0,057	0,060		0,0585	0,0021	0,0469			115%	
	MP UVox coconut charcoal (EtOH)	4	0,060	0,056		0,0580	0,0028	0,0469			114%	
	MP UVox coconut charcoal (EtOH)	3	0,063	0,060		0,0615	0,0021	0,0469			121%	
	MP UVox coconut charcoal (EtOH)	2	0,056	0,053		0,0545	0,0021	0,0469			108%	

using formula for TA98+S9 by lack of sufficient historic NC data TA100

TA100 +S9			cytotoxicity response					statistics cytotoxicity			
			OD at 595 nm								
vial code	sample name	dose (uL)	well 1	well 2	well 3	average	stdev	DL	x < DL?	% cytotoxic	cytotoxic?
	NC	6	0,061	0,055	0,059	0,0583	0,0031	0,0518		100%	
	PC	6	0,063	0,064	0,063	0,0633	0,0006	0,0518		109%	
	PC cytotox	6	0,049	0,041	0,040	0,0433	0,0049	0,0518	Yes	74%	Yes
	PrC Oasis HLB	6	0,051	0,055	0,054	0,0533	0,0021	0,0518		91%	
	MP UVox Oasis HLB (DMSO)	6				#DIV/0!	#DIV/0!	0,0518	#DIV/0!	#DIV/0!	
	MP UVox Oasis HLB (DMSO)	5	0,061	0,066		0,0635	0,0035	0,0518		109%	
	MP UVox Oasis HLB (DMSO)	4				#DIV/0!	#DIV/0!	0,0518	#DIV/0!	#DIV/0!	
	MP UVox Oasis HLB (DMSO)	3				#DIV/0!	#DIV/0!	0,0518	#DIV/0!	#DIV/0!	
	MP UVox Oasis HLB (DMSO)	2				#DIV/0!	#DIV/0!	0,0518	#DIV/0!	#DIV/0!	
	NC Ethanol	6	0,054	0,062	0,047	0,0543	0,0075	0,0478		100%	
	PrC XAD pH neutral	6	0,051	0,055	0,046	0,0507	0,0045	0,0478		93%	
	PrC XAD pH 2	6	0,058	0,051	0,051	0,0533	0,0040	0,0478		98%	
	PrC Oasis HLB	6	0,056	0,051	0,053	0,0533	0,0025	0,0478		98%	
	PrC coconut charcoal	6	0,049	0,049	0,058	0,0520	0,0052	0,0478		96%	
	MP UVox XAD pH neutral	6	0,049	0,045		0,0470	0,0028	0,0478	Yes	87%	Yes
	MP UVox XAD pH neutral	5	0,068	0,046		0,0570	0,0156	0,0478		105%	
	MP UVox XAD pH neutral	4	0,043	0,044		0,0435	0,0007	0,0478	Yes	80%	Yes
	MP UVox XAD pH neutral	3	0,045	0,047		0,0460	0,0014	0,0478	Yes	85%	Yes
	MP UVox XAD pH neutral	2	0,045	0,048		0,0465	0,0021	0,0478	Yes	86%	Yes
	MP UVox XAD pH 2	6	0,049	0,049		0,0490	0,0000	0,0478		90%	
	MP UVox XAD pH 2	5	0,052	0,053		0,0525	0,0007	0,0478		97%	
	MP UVox XAD pH 2	4	0,048	0,054		0,0510	0,0042	0,0478		94%	
	MP UVox XAD pH 2	3	0,052	0,061		0,0565	0,0064	0,0478		104%	
	MP UVox XAD pH 2	2	0,069	0,064		0,0665	0,0035	0,0478		122%	
	MP UVox Oasis HLB (EtOH)	6	0,055	0,059		0,0570	0,0028	0,0478		105%	
	MP UVox Oasis HLB (EtOH)	5	0,056			0,0560	#DIV/0!	0,0478		103%	
	MP UVox Oasis HLB (EtOH)	4	0,060			0,0600	#DIV/0!	0,0478		110%	
	MP UVox Oasis HLB (EtOH)	3				#DIV/0!	#DIV/0!	0,0478	#DIV/0!	#DIV/0!	
	MP UVox Oasis HLB (EtOH)	2				#DIV/0!	#DIV/0!	0,0478	#DIV/0!	#DIV/0!	
	MP UVox coconut charcoal (EtOH)	6				#DIV/0!	#DIV/0!	0,0478	#DIV/0!	#DIV/0!	
	MP UVox coconut charcoal (EtOH)	5	0,050	0,061		0,0555	0,0078	0,0478		102%	
	MP UVox coconut charcoal (EtOH)	4	0,046	0,056		0,0510	0,0071	0,0478		94%	
	MP UVox coconut charcoal (EtOH)	3	0,051			0,0510	#DIV/0!	0,0478		94%	
	MP UVox coconut charcoal (EtOH)	2	0,052	0,048		0,0500	0,0028	0,0478		92%	

using formula for TA98+S9 by lack of sufficient historic NC data TA100

Genotoxicity data:

TA98 -S9			genotoxicity response						statistics genotoxicity	
			number of yellow wells (of 48)							genotoxic?
vial code	sample name	dose (uL)	well 1	well 2	well 3	sum	average	stdev	DL	(sum>DL?)
	NC (DMSO)	6	3	1	1	5	1,67	1,15	11	
	PC	6	37	31	34	102	34,00	3,00		Yes
	PC cytotox	6	0	0	0	0	0,00	0,00		
08-10-2010/**25**	PrC Oasis HLB (DMSO)	6	0	1	0	1	0,33	0,58		
08-10-2010/**7**	MP UVox Oasis HLB (DMSO)	6	44	43		130,5	43,50	0,71		Yes
	MP UVox Oasis HLB (DMSO)	5	43	37		120	40,00	4,24		Yes
	MP UVox Oasis HLB (DMSO)	4	40	39		118,5	39,50	0,71		Yes
	MP UVox Oasis HLB (DMSO)	3	35	32		100,5	33,50	2,12		Yes
	MP UVox Oasis HLB (DMSO)	2	23	24		70,5	23,50	0,71		Yes
	NC (EtOH)	6	1	1	1	3	1,00	0,00	8	
08-10-2010/**3**	PrC XAD pH neutral	6	1	2	1	4	1,33	0,58		
08-10-2010/**5**	PrC XAD pH 2	6	1	2	4	7	2,33	1,53		
08-10-2010/**27**	PrC Oasis HLB (EtOH)	6	2	4	4	10	3,33	1,15		Yes
08-10-2010/**43**	PrC coconut charcoal	6	3	2	2	7	2,33	0,58		
03-09-2010/**98**	MP UVox XAD pH neutral	6	36	43		118,5	39,50	4,95		Yes
	MP UVox XAD pH neutral	5	41	33		111	37,00	5,66		Yes
	MP UVox XAD pH neutral	4	31	34		97,5	32,50	2,12		Yes
	MP UVox XAD pH neutral	3	31	31		93	31,00	0,00		Yes
	MP UVox XAD pH neutral	2	24	19		64,5	21,50	3,54		Yes
08-10-2010/**1**	MP UVox XAD pH 2	6	32	32		96	32,00	0,00		Yes
	MP UVox XAD pH 2	5	24	33		85,5	28,50	6,36		Yes
	MP UVox XAD pH 2	4	23	30		79,5	26,50	4,95		Yes
	MP UVox XAD pH 2	3	28	23		76,5	25,50	3,54		Yes
	MP UVox XAD pH 2	2	15	14		43,5	14,50	0,71		Yes
08-10-2010/**9**	MP UVox Oasis HLB (EtOH)	6	36	37		109,5	36,50	0,71		Yes
	MP UVox Oasis HLB (EtOH)	5	36	32		102	34,00	2,83		Yes
	MP UVox Oasis HLB (EtOH)	4	39	37		114	38,00	1,41		Yes
	MP UVox Oasis HLB (EtOH)	3	32	39		106,5	35,50	4,95		Yes
	MP UVox Oasis HLB (EtOH)	2	23	27		75	25,00	2,83		Yes
08-10-2010/**42**	MP UVox coconut charcoal (EtOH)	6	2	1		4,5	1,50	0,71		
	MP UVox coconut charcoal (EtOH)	5	3	2		7,5	2,50	0,71		
	MP UVox coconut charcoal (EtOH)	4	0	5		7,5	2,50	3,54		
	MP UVox coconut charcoal (EtOH)	3	3	0		4,5	1,50	2,12		
	MP UVox coconut charcoal (EtOH)	2	2	2		6	2,00	0,00		

sum of duplicatie is multiplied by 1,5 to extrapolate to triplicate

TA98 +S9			genotoxicity response						statistics genotoxicity	
			number of yellow wells (of 48)							genotoxic?
vial code	sample name	dose (uL)	well 1	well 2	well 3	sum	average	stdev	DL	(sum>DL?)
	NC (DMSO)	6	1	0	1	2	0,67	0,58	6	
	PC	6	32	36	34	102	34,00	2,00		Yes
	PC cytotox	6	1	0	0	1	0,33	0,58		
08-10-2010/**25**	PrC Oasis HLB (DMSO)	6	2	5	0	7	2,33	2,52		Yes
08-10-2010/**7**	MP UVox Oasis HLB (DMSO)	6	32	32		96	32,00	0,00		Yes
	MP UVox Oasis HLB (DMSO)	5	31	32		94,5	31,50	0,71		Yes
	MP UVox Oasis HLB (DMSO)	4	25	23		72	24,00	1,41		Yes
	MP UVox Oasis HLB (DMSO)	3	23	16		58,5	19,50	4,95		Yes
	MP UVox Oasis HLB (DMSO)	2	15	11		39	13,00	2,83		Yes
	NC (EtOH)	6	2	0	1	3	1,00	1,00	8	
08-10-2010/**3**	PrC XAD pH neutral	6	0	1	1	2	0,67	0,58		
08-10-2010/**5**	PrC XAD pH 2	6	1	2	4	7	2,33	1,53		
08-10-2010/**27**	PrC Oasis HLB (EtOH)	6	1	3	2	6	2,00	1,00		
08-10-2010/**43**	PrC coconut charcoal	6	1	3	1	5	1,67	1,15		
03-09-2010/**98**	MP UVox XAD pH neutral	6	29	22		76,5	25,50	4,95		Yes
	MP UVox XAD pH neutral	5	24	23		70,5	23,50	0,71		Yes
	MP UVox XAD pH neutral	4	22	20		63	21,00	1,41		Yes
	MP UVox XAD pH neutral	3	18	17		52,5	17,50	0,71		Yes
	MP UVox XAD pH neutral	2	12	8		30	10,00	2,83		Yes
08-10-2010/**1**	MP UVox XAD pH 2	6	25	27		78	26,00	1,41		Yes
	MP UVox XAD pH 2	5	24	16		60	20,00	5,66		Yes
	MP UVox XAD pH 2	4	21	14		52,5	17,50	4,95		Yes
	MP UVox XAD pH 2	3	21	14		52,5	17,50	4,95		Yes
	MP UVox XAD pH 2	2	6	9		22,5	7,50	2,12		Yes
08-10-2010/**9**	MP UVox Oasis HLB (EtOH)	6	38	37		112,5	37,50	0,71		Yes
	MP UVox Oasis HLB (EtOH)	5	36	37		109,5	36,50	0,71		Yes
	MP UVox Oasis HLB (EtOH)	4	34	35		103,5	34,50	0,71		Yes
	MP UVox Oasis HLB (EtOH)	3	27	33		90	30,00	4,24		Yes
	MP UVox Oasis HLB (EtOH)	2	30	24		81	27,00	4,24		Yes
08-10-2010/**42**	MP UVox coconut charcoal (EtOH)	6	2	3		7,5	2,50	0,71		
	MP UVox coconut charcoal (EtOH)	5	2	2		6	2,00	0,00		
	MP UVox coconut charcoal (EtOH)	4	2	1		4,5	1,50	0,71		
	MP UVox coconut charcoal (EtOH)	3	0	0		0	0,00	0,00		
	MP UVox coconut charcoal (EtOH)	2	1	2		4,5	1,50	0,71		

sum of duplicatie is multiplied by 1,5 to extrapolate to triplicate

TA100 -S9			genotoxicity response						statistics genotoxicity	
			number of yellow wells (of 48)							genotoxic?
vial code	sample name	dose (uL)	well 1	well 2	well 3	sum	average	stdev	DL	(sum>DL?)
	NC (DMSO)	6	10	11	7	28	9,33	2,08	39	
	PC	6	48	46	48	142	47,33	1,15		Yes
	PC cytotox	6	0	0	0	0	0,00	0,00		
08-10-2010/**25**	PrC Oasis HLB (DMSO)	6	10	8	9	27	9,00	1,00		
08-10-2010/**7**	MP UVox Oasis HLB (DMSO)	6	45			135	45,00	#DIV/0!		Yes
	MP UVox Oasis HLB (DMSO)	5	44	44		132	44,00	0,00		Yes
	MP UVox Oasis HLB (DMSO)	4	38	41		118,5	39,50	2,12		Yes
	MP UVox Oasis HLB (DMSO)	3	38	38		114	38,00	0,00		Yes
	MP UVox Oasis HLB (DMSO)	2	34	31		97,5	32,50	2,12		Yes
	NC (EtOH)	6	13	11	12	36	12,00	1,00	48	
08-10-2010/**3**	PrC XAD pH neutral	6	7	13	12	32	10,67	3,21		
08-10-2010/**5**	PrC XAD pH 2	6	13	8	9	30	10,00	2,65		
08-10-2010/**27**	PrC Oasis HLB (EtOH)	6	10	11	15	36	12,00	2,65		
08-10-2010/**43**	PrC coconut charcoal	6	10	15	13	38	12,67	2,52		
03-09-2010/**98**	MP UVox XAD pH neutral	6	48	45		139,5	46,50	2,12		Yes
	MP UVox XAD pH neutral	5	42	46		132	44,00	2,83		Yes
	MP UVox XAD pH neutral	4	45	46		136,5	45,50	0,71		Yes
	MP UVox XAD pH neutral	3	41	44		127,5	42,50	2,12		Yes
	MP UVox XAD pH neutral	2	32	30		93	31,00	1,41		Yes
08-10-2010/**1**	MP UVox XAD pH 2	6	46	48		141	47,00	1,41		Yes
	MP UVox XAD pH 2	5	47	44		136,5	45,50	2,12		Yes
	MP UVox XAD pH 2	4	36	43		118,5	39,50	4,95		Yes
	MP UVox XAD pH 2	3	40	38		117	39,00	1,41		Yes
	MP UVox XAD pH 2	2	28	27		82,5	27,50	0,71		Yes
08-10-2010/**9**	MP UVox Oasis HLB (EtOH)	6	47	47		141	47,00	0,00		Yes
	MP UVox Oasis HLB (EtOH)	5	46	45		136,5	45,50	0,71		Yes
	MP UVox Oasis HLB (EtOH)	4	46	44		135	45,00	1,41		Yes
	MP UVox Oasis HLB (EtOH)	3	45	42		130,5	43,50	2,12		Yes
	MP UVox Oasis HLB (EtOH)	2	35			105	35,00	#DIV/0!		Yes
08-10-2010/**42**	MP UVox coconut charcoal (EtOH)	6	9	14		34,5	11,50	3,54		
	MP UVox coconut charcoal (EtOH)	5	7	13		30	10,00	4,24		
	MP UVox coconut charcoal (EtOH)	4	6	7		19,5	6,50	0,71		
	MP UVox coconut charcoal (EtOH)	3	13	15		42	14,00	1,41		
	MP UVox coconut charcoal (EtOH)	2	10	9		28,5	9,50	0,71		

sum of duplicatie is multiplied by 1,5 to extrapolate to triplicate

TA100 +S9			genotoxicity response						statistics genotoxicity	
			number of yellow wells (of 48)							genotoxic?
vial code	sample name	dose (uL)	well 1	well 2	well 3	sum	average	stdev	DL	(sum>DL?)
	NC (DMSO)	6	7	5	3	15	5,00	2,00	24	
	PC	6	48	48	48	144	48,00	0,00		Yes
	PC cytotox	6	0	0	0	0	0,00	0,00		
08-10-2010/**25**	PrC Oasis HLB (DMSO)	6	4	7	6	17	5,67	1,53		
08-10-2010/**7**	MP UVox Oasis HLB (DMSO)	6				0	#DIV/0!	#DIV/0!		
	MP UVox Oasis HLB (DMSO)	5	45	45		135	45,00	0,00		Yes
	MP UVox Oasis HLB (DMSO)	4				0	#DIV/0!	#DIV/0!		
	MP UVox Oasis HLB (DMSO)	3				0	#DIV/0!	#DIV/0!		
	MP UVox Oasis HLB (DMSO)	2				0	#DIV/0!	#DIV/0!		
	NC (EtOH)	6	2	1	12	15	5,00	6,08	24	
08-10-2010/**3**	PrC XAD pH neutral	6	11	6	3	20	6,67	4,04		
08-10-2010/**5**	PrC XAD pH 2	6	10	11	7	28	9,33	2,08		Yes
08-10-2010/**27**	PrC Oasis HLB (EtOH)	6	8	8	14	30	10,00	3,46		Yes
08-10-2010/**43**	PrC coconut charcoal	6	3	7	4	14	4,67	2,08		
03-09-2010/**98**	MP UVox XAD pH neutral	6	26	37		94,5	31,50	7,78		Yes
	MP UVox XAD pH neutral	5	29	27		84	28,00	1,41		Yes
	MP UVox XAD pH neutral	4	24	31		82,5	27,50	4,95		Yes
	MP UVox XAD pH neutral	3	23	18		61,5	20,50	3,54		Yes
	MP UVox XAD pH neutral	2	16	19		52,5	17,50	2,12		Yes
08-10-2010/**1**	MP UVox XAD pH 2	6	43	43		129	43,00	0,00		Yes
	MP UVox XAD pH 2	5	42	43		127,5	42,50	0,71		Yes
	MP UVox XAD pH 2	4	43	45		132	44,00	1,41		Yes
	MP UVox XAD pH 2	3	28	29		85,5	28,50	0,71		Yes
	MP UVox XAD pH 2	2	14	13		40,5	13,50	0,71		Yes
08-10-2010/**9**	MP UVox Oasis HLB (EtOH)	6	43	42		127,5	42,50	0,71		Yes
	MP UVox Oasis HLB (EtOH)	5	44			132	44,00	#DIV/0!		Yes
	MP UVox Oasis HLB (EtOH)	4	45			135	45,00	#DIV/0!		Yes
	MP UVox Oasis HLB (EtOH)	3				0	#DIV/0!	#DIV/0!		
	MP UVox Oasis HLB (EtOH)	2				0	#DIV/0!	#DIV/0!		
08-10-2010/**42**	MP UVox coconut charcoal (EtOH)	6				0	#DIV/0!	#DIV/0!		
	MP UVox coconut charcoal (EtOH)	5	5	2		10,5	3,50	2,12		
	MP UVox coconut charcoal (EtOH)	4	4	0		6	2,00	2,83		
	MP UVox coconut charcoal (EtOH)	3	5			15	5,00	#DIV/0!		
	MP UVox coconut charcoal (EtOH)	2	2	2		6	2,00	0,00		

sum of duplicatie is multiplied by 1,5 to extrapolate to triplicate

VIII Raw data of comet assay in comparison study

HepG2 after 3 h		genotoxicity response mean tail intensity per culture				statistics genotoxicity	
vial code	sample name	culture 1	culture 2	average	stdev	fold increase	genotoxic? (>3?)
	NC (EtOH)	2,87	3,07	3,0	0,1		
	PC	49,73	45,21	47,5	3,2	16,0	Yes
08-10-2010/**4**	PrC XAD-4 pH 7 oct '10	2,88	2,55	2,7	0,2	0,9	
08-10-2010/**6**	PrC XAD-4 pH 2 oct '10	3,39	4,36	3,9	0,7	1,3	
03-09-2010/**99**	MP UVox XAD-4 pH 7 oct '10	3,02	4,1	3,6	0,8	1,2	
08-10-2010/**2**	MP UVox XAD-4 pH 2 oct '10	3,87	4,35	4,1	0,3	1,4	
10-01-2011/**9**	PrC XAD-4 pH 7 march '11	2,91	3,75	3,3	0,6	1,1	
10-01-2011/**10**	PrC XAD-4 pH 2 march '11	3,35	2,33	2,8	0,7	1,0	
21-03-2011/**13**	PrC XAD-2/8 pH 2 march '11	2,39	1,76	2,1	0,4	0,7	
21-03-2011/**11**	MP UVox XAD-4 pH 7 march '11	1,54	1,74	1,6	0,1	0,6	
21-03-2011/**12**	MP UVox XAD-4 pH 2 march '11	2,5	2,5	2,5	0,0	0,8	
21-03-2011/**14**	MP UVox XAD-2/8 pH 2 march '11	3,38	1,58	2,5	1,3	0,8	
	NC (DMSO)	2,78	3,09	2,9	0,2		
08-10-2010/**26**	PrC Oasis HLB oct '10	3,49	2,17	2,8	0,9	1,0	
08-10-2010/**8**	MP UVox Oasis HLB pH 2 oct '10	2,93	2,68	2,8	0,2	1,0	

HepG2 after 24 h		genotoxicity response mean tail intensity per culture				statistics genotoxicity	
vial code	sample name	plate 1	plate 2	average	stdev	fold increase	genotoxic? (>3?)
	NC (EtOH)	10,59	2,89	6,7	5,4		
	PC	52,47	49,71	51,1	2,0	7,6	Yes
08-10-2010/**4**	PrC XAD-4 pH 7 oct '10	4	3,29	3,6	0,5	0,5	
08-10-2010/**6**	PrC XAD-4 pH 2 oct '10	1,6	6,33	4,0	3,3	0,6	
03-09-2010/**99**	MP UVox XAD-4 pH 7 oct '10	2,33	1,86	2,1	0,3	0,3	
08-10-2010/**2**	MP UVox XAD-4 pH 2 oct '10	2,65	3,93	3,3	0,9	0,5	
10-01-2011/**9**	PrC XAD-4 pH 7 march '11	1,69	1,56	1,6	0,1	0,2	
10-01-2011/**10**	PrC XAD-4 pH 2 march '11	1,57	2,56	2,1	0,7	0,3	
21-03-2011/**13**	PrC XAD-2/8 pH 2 march '11	2,54	2,72	2,6	0,1	0,4	
21-03-2011/**11**	MP UVox XAD-4 pH 7 march '11	2,54	1,9	2,2	0,5	0,3	
21-03-2011/**12**	MP UVox XAD-4 pH 2 march '11	4,13	2,41	3,3	1,2	0,5	
21-03-2011/**14**	MP UVox XAD-2/8 pH 2 march '11	1,81	1,92	1,9	0,1	0,3	
	NC (DMSO)	1,69	1,9	1,8	0,1		
08-10-2010/**26**	PrC Oasis HLB oct '10	2,32	2,47	2,4	0,1	1,3	
08-10-2010/**8**	MP UVox Oasis HLB pH 2 oct '10	1,95	2,25	2,1	0,2	1,2	

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