Toxicology of Chemical Dispersants in Alaskan Whales

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John Pierce Wise, Sr., Ph.D.

University of Southern Maine

96 Falmouth St.

PO Box 9300

Portland, ME 04104-9300

Phone (207) 228-8050

Email: john.wise@usm.maine.edu

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Abstract

There have been two major oil crises in United States history, the 1989 Exxon-Valdez oil spill in Alaska and the 2010 Deepwater Horizon Oil Rig explosion in the Gulf of Mexico. The aftermath of both of these events resulted in immediate and severe impacts on wildlife and humans. However, there is still not a full understanding of the long term effects these spills had on wildlife. These events drew attention to the need for toxicological experiments to better understand the impact of oil and the chemicals used to disperse the oil on wildlife species. Of particular interest are the effects on whales as they are air breathing, warm-blooded mammals that nurse their young and can most closely represent humans in the ocean. Whales are important species in food webs, are one of the major bases of ecotourism, are charismatic species that capture the attention of the public at large and can integrate all possible routes of exposure to dispersants in the environment (air, water and food). Consequently, they make excellent models to use for studying the threats and consequences of oil and dispersant exposure. There is very little laboratory-based data regarding the toxicity of these substances in marine mammals. Thus we sought to determine the toxicity of Alaskan oil, dispersants, and chemically dispersed oil in whales. We found that dispersants are cytotoxic and genotoxic to sperm whales but not genotoxic to humpback cells. In addition, we found that oil induced genotoxic effects in whale cells and adding dispersants increased the toxicity of the oil.

Introduction

On April 20, 2010, the Deepwater Horizon oil-rig exploded resulting in an uncontrolled release of oil into the Gulf of Mexico. The rig was located about 50 miles offshore in more than 5,000 feet of water. By the time the well was capped and the oil flow stopped, approximately

200 million gallons of oil were released into the Gulf and the oil spill had become the worst in U.S. history and one of the worst in world history.

Oil washed onto Gulf coastlines and fouled inshore waters threatening commercially valuable fisheries, fueling public outrage and severely impacting the tourism, fishing and oil industries, all during one of the worst economic periods in U.S. history. To address this problem, British Petroleum (BP), the company responsible for the well, administered at least 2 million gallons of chemical dispersants by either injecting it deep into the water or spraying it on the surface (Kujawinski et al., 2011). This approach was unprecedented and was the first use of these chemicals on such a massive scale.

The intent of the dispersant application was straightforward - breakup the oil into smaller droplets to remove it from the surface and cause it to sink so currents could carry it elsewhere. The expectation was that the oil would become diluted by the vast ocean waters. The consequence of the dispersant exposure was equally straightforward. The oil would remain offshore longer and would be mobilized throughout the water column instead of just sitting on the surface, thus increasing the potential exposure to offshore species. In other words, the dispersants would increase the exposure and potential toxicity to offshore species in an effort to decrease the exposure and potential toxicity to coastal areas and inshore waters.

The toxicity of the dispersants to marine life is poorly understood and is understudied (Wise and Wise 2011). The vast majority of studies are limited to genocidal-type endpoints i.e. how much dispersant in necessary to kill 50% of a population. Very few consider more subtle endpoints such as behavioral effects and none have considered possible DNA damaging effects. Even fewer studies consider the toxicity of dispersants mixed with oil and none have considered dispersants mixed with the toxic metals found in oil. Thus, the Gulf of Mexico became the largest marine toxicology experiment ever.

Since the crisis and the application of the dispersants, the crude oil has disappeared from the surface of the water causing negative public pressure and attention to end. The significant success of removing oil from view and relieving public pressure has created an atmosphere where dispersant application is likely to become the first and primary response to future oil spills. However, the toxicity of the dispersants and the dispersed oil remains poorly understood. It is still unclear that from a scientific and health point of view whether application of dispersants was successful or not. States like Alaska and Maine and others with large oil-related activity face significant risks of large oil spills. Thus, it is essential we learn the necessary lessons about these chemicals and chemical mixtures.

This project sought to use this crisis as a case study of oil pollution and understand the impacts of chemical dispersants, crude oil and dispersed oil on the health of marine life. We focus on the impacts of these agents on DNA. All life depends on its DNA. DNA damage is well understood to be a short-term test for a myriad of diseases and negative health outcomes including both cancer and reproductive toxicity. DNA damage is a required hazard characterization by the U.S. Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) as part of their risk assessments. Thus, it is an important and appropriate starting point for understanding dispersant toxicity.

The results of this project help to further characterize the potential impact of dispersants and dispersed oil on marine life so that better policies and procedures can be developed to protect against future spills and to better understand ocean pollution. We used whales as model marine species. As air breathing, warm-blooded mammals that nurse their young, whales and their marine mammal relatives represent humans most closely in the ocean. Whales are important species in food webs, are one of the major bases of ecotourism, are charismatic species that capture the attention of the public at large and can integrate all possible routes of exposure to dispersants in the environment (air, water and food). Consequently, they make

excellent models to use for studying the threats and consequences of dispersant exposure. We used whale cells to determine the impacts on whale DNA. The central hypothesis, that dispersants and dispersed oil can damage whale DNA was accomplished with following two objectives:

Objective 1. Measure the Cytotoxicity of Chemical Dispersants and Dispersed Oil on Whale Cells.

Cytotoxicity is the ability of an agent to induce cell death. The cytotoxic effects of chemical dispersants and dispersed oil are uncertain. This objective will determine the cytotoxic effects of these agents in whale cells using assays for cell survival.

Objective 2. Determine the Genotoxicity of Chemical Dispersants and Dispersed Oil on Whale DNA.

Genotoxicity is the ability of an agent to damage DNA. The genotoxic effects of chemical dispersants and dispersed oil are unknown. This objective will determine the genotoxic effects of these agents in whale cells with assays for DNA strand breaks and chromosomal aberrations.

Materials and Methods

A critical part of assessing the risk posed by a chemical is to understand its potential to damage DNA, as genotoxic (i.e. DNA damaging) chemicals are likely to be carcinogenic and interfere with embryogenesis and development. To assess the impact of dispersants and dispersed oil on marine life DNA, we treated whale cell lines with oil, dispersants, dispersed oil,

and oil-related metals to determine if they can induce cell death and DNA damage in whale cells and if so how much it takes to do so.

Whale Cell Culture Models

Whale cell lines are difficult to obtain and is an area that the Wise Laboratory has been pioneering. We have developed the only cell lines for humpback (*Megaptera novaeangliae*) and sperm (*Physeter macrocephalus*) whales and both are primary skin fibroblasts obtained from apparently health, free-ranging whales. We used these cells to test the toxicity of dispersants for both of these species. We chose these two species as they represent different ecological niches. Humpbacks occupy more sheltered waterways and are baleen feeders consuming krill and small fish. Sperm whales are more offshore and are toothed whales feeding on squid and larger fish. Both are Alaskan species.

All cells were grown in DMEM/F-12 medium containing 15% Cosmic calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 ug/ml streptomycin, and 0.1 mM sodium pyruvate. All whale cells were maintained in a 33ºC, humidified incubator with 5% CO_2 and routinely tested for mycoplasma contamination.

Working with marine mammals requires a federal permit from the National Marine Fisheries Service (NMFS). This requirement extends from contact with the animals themselves to cells, DNA, RNA and protein. The Wise Laboratory has a generous permit from NMFS to obtain marine mammal tissue, develop cell lines and study cellular and molecular events including DNA, RNA and protein. Cell line and contaminant measurements are covered by NMFS permit # 16305 (J. Wise, PI).

Chemical Dispersants, Oil and Dispersed Oil Treatments

We determined the toxic effects of chemical dispersants, crude oil and dispersed crude oil in the whale cells. This provided information on the toxicity of each as well as comparisons between them to see if dispersants altered the toxicity of the crude oil. We obtained crude oil from Alaska from Prince William Sound Regional Citizens' Advisory Council (PWSRCAC). We also obtained samples of the two major dispersants used in the Deepwater Horizon crisis, Corexit 9527 and Corexit 9500, as a generous gift from Nalco Holding Company, the manufacturer. Whale cells were exposed for 24 hours to Corexit 9527, Corexit 9500, the water-accommodated fraction of oil, or dispersed oil. These are organic chemicals and, therefore, were studied under low light conditions with and without an S9 fraction to ensure metabolism.

Chemical Dispersants

Treatment dilutions of Corexit 9527 and Corexit 9500 were prepared under dark conditions using the 100% stock solution and sterile water. Final concentrations of dispersant were expressed as a percent of the total volume of extracellular media.

Water Accommodated Fraction of Crude Oil and Chemically Enhanced Water Accommodated Fraction of Crude Oil

The water accommodated fraction (WAF) of crude oil and chemically enhanced water accommodated fraction (CEWAF) of crude oil were prepared using the Hodson Lab SOP, which followed the method of Singer et al. 2000. Briefly, 10 ml of Alaskan crude oil and 90 ml of water (1:10 oil:water ratio) were added into a 250 ml side-arm flask. The flask was placed on a magnetic stir plate with a 0.5 in Teflon coated magnetic stir bar. The WAF was spun for 18 h in the dark,

with speed such that the vortex formed in the oil was 1/3 the height of the water. After 18 h the stir plate was turned off and the oil/water mixture was allowed to settle for 1 h; the bottom layer of the WAF was collected. CEWAF was prepared the same way as WAF, with the exception that after 18 h of stirring, 1 ml of Corexit® 9527 (Nalco Holding Company, Naperville, Illinois) was added. After 1 h of additional stirring, the stir plate was shut off and the CEWAF was allowed to settle for 1 hour, after which time the bottom layer was collected in the same way as the WAF. Cells were treated with WAF and CEWAF based on a percent of total volume of extracellular media, concentrations of 0, 0.5, 1, 5, 10 or 20% of the total volume were used.

S9 Fractions

Although the metabolism of the dispersants is unknown, we considered phase 1 metabolism to determine if there was a difference in toxicity between the metabolite and the parent compound. Since fibroblast cells may not express cytochrome P450s to metabolize organic compounds we induced phase 1 metabolism using S9 fractions to compare to the parent compound. S9 fractions were prepared with 1X Tris buffer, NADPH regenerating system solution A, NADPH regenerating system solution B, and liver S9 fractions. The mixture was prepared just prior to each individual treatment and applied at the same time as the compound of interest. Cultures were treated with and without S9 co-treatment for comparison. Sodium chromate served as a positive control for all experiments. It was dissolved in water and filter sterilized.

Cytotoxicity

We used a clonogenic assay based on our published methods to determine the cytotoxicity of each dispersant (Wise et al., 2011). Briefly, cells were seeded into two 6 well tissue culture plates and allowed 48 h to resume normal log phase growth. Then they were treated with Corexit 9500, Corexit 9527, WAF or CEWAF for 24 h. All treatment doses were done with and without S9 fractions. After the treatment time cells were reseeded into gelatin coated 100 mm tissue culture dishes at colony forming density. Once adequate cell colonies formed (~2 weeks) dishes were rinsed twice with 1X phosphate-buffered saline (PBS) then fixed in methanol for 20 min and stained with crystal violet stain for 30 min. Dishes were analyzed for number of colonies formed, treated dishes were compared to the negative control.

Genotoxicity

We used a chromosomal aberration assay to determine the genotoxicity of each chemical, based on our published methods (Wise et al., 2011). Briefly, cells were seeded into 100 mm tissue culture dishes for 48 h. Then they were treated with either Corexit 9500, Corexit 9527, WAF or CEWAF for 24 h. All treatment doses were done with and without S9 fractions. Five hours prior to the end of the treatment period cells were arrested in metaphase using 0.1 ug/ml demecolcine solution. After the full 24 h treatment period, cells were resuspended in a potassium chloride hypotonic solution (KCl) for 17 min then fixed with 3:1 methanol:acetic acid. After two changes of fixative, cells were dropped onto microscope slides and stained with 5% Giemsa stain in Gurr's Buffer. Slides were analyzed for chromosome aberrations in 100 metaphases per treatment concentration according to our published methods (Wise et al., 2011).

Chemical Analysis

In order to better understand the cellular PAH exposures in our experimental system, we analyzed samples of the WAF and CEWAF preparations. Multiple analyses were performed on the solutions: samples were taken from the WAF and CEWAF preparations at each treatment time to assess differences between preparations and potential changes in each preparation over time; the extracellular media after treatment at multiple time point to assess changes in the extracellular PAHs over the treatment period (0-24 h); the extracellular media after different treatment concentrations to quantitate the dilution series. In addition, a media blank series was collected to account for any background chemicals as a result of the media and plastic cultureware. All chemical analyses were performed by AXYS Analytical Services Ltd.

Statistics

The statistical difference between values for cytotoxicity and genotoxicity were evaluated using t-test and multiple regression analysis with treatment, concentration, and S9 as the three independent variables. No adjustment was made for multiple comparisons.

Results

Corexit 9500 and 9527 Cytotoxicity in Sperm Whale Skin Cells

Corexit 9500 induced a concentration-dependent increase in cytotoxicity in sperm whale skin cells (Figure 1). Concentrations of 0.005, 0.025, 0.05 and 0.1 percent 9500 induced 82, 67, 25 and 3 percent relative survival, respectively. S9 mediated metabolism did not significantly

alter the cytotoxicity of Corexit 9500 (Figure 1). Concentrations of 0.005, 0.025, 0.05 and 0.1 percent 9500 with S9 fractions induced 91, 72, 37 and 3 percent relative survival, respectively.

Corexit 9527 also induced a concentration dependent increase in cytotoxicity (Figure 2). Concentrations of 0.005, 0.025, 0.05 and 0.1 percent 9527 induced 86, 79, 70 and 26 percent relative survival, respectively. S9 mediated metabolism resulted in a similar dose response (Figure 2). Concentrations of 0.005, 0.025, 0.05 and 0.1 percent 9527 with S9 fractions induced 86, 77, 70 and 42 percent relative survival, respectively.

Comparison of Corexit 9500 and Corexit 9527 shows that 9500 is more toxic than 9527 (Figure 3) to sperm whale skin cells. For example, there is a 3-fold increase in toxicity at 0.05 percent concentration ($p = 0.0002$).

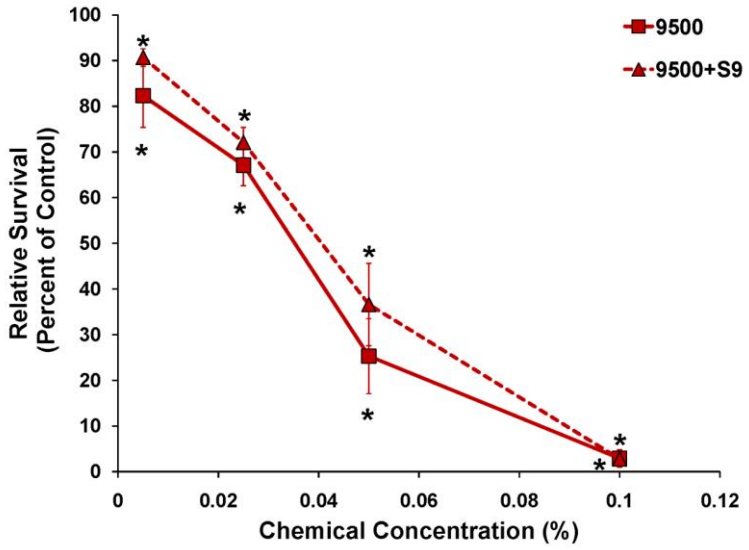

Figure 1. Corexit 9500 is cytotoxic to sperm whale skin cells. This figure shows that Corexit 9500 was cytotoxic to sperm whale skin cells after a 24 h exposure and S9 mediated metabolism did not alter cytotoxicity. Cytotoxicity is measured as cell survival relative to the control. The overall dose-response is highly significant ($p < 0.0001$). There was no statistical difference observed between 9500 and 9500+ S9 ($p = 0.19$). Data represent 3-4 experiments ± the standard error of the mean. *indicates doses that are significantly different from control ($p < 0.05$).

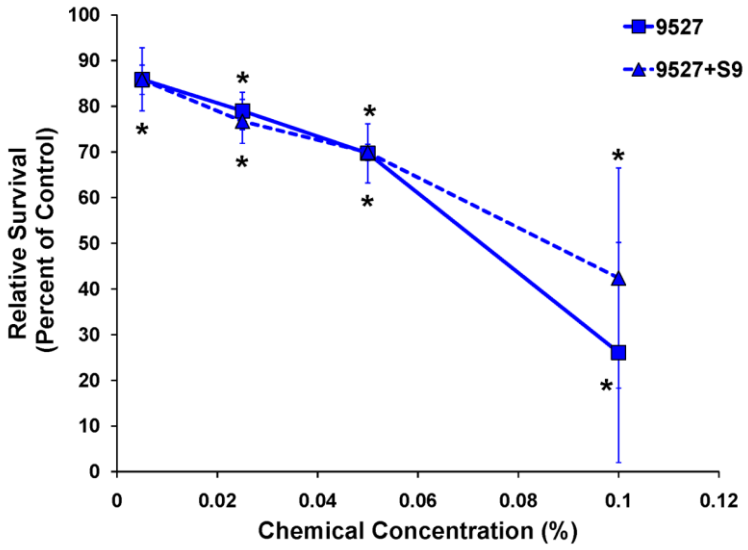

Figure 2. Corexit 9527 is cytotoxic to sperm whale skin cells. This figure shows Corexit 9527 was cytotoxic to sperm whale skin cells in a concentration dependent manner after a 24 h and S9 mediated metabolism did not alter cytotoxicity. Cytotoxicity is measured as cell survival relative to the control. There was no statistical difference observed between 9527 and 9527+ S9 ($p > 0.05$). Data represent 3 experiments ± the standard error of the mean. *indicates doses that are statistically significant from control ($p < 0.05$).

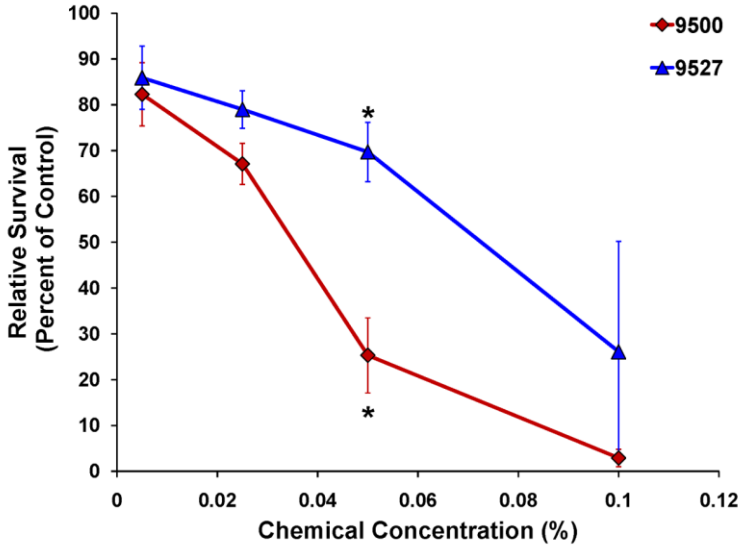

Figure 3. Corexit 9500 is more cytotoxic than Corexit 9527 in sperm whale skin cells. This figure compares the cytotoxicity of Corexit 9500 and Corexit 9527 after a 24 h exposure. Sperm whale cells were more sensitive to Corexit 9500 than Corexit 9527 ($p = 0078$). *indicates doses that were significantly different from each other ($p < 0.05$).

Corexit 9500 and 9527 Cytotoxicity in Humpback Whale Skin Cells

Corexit 9500 induced a concentration-dependent increase in cytotoxicity to humpback whale skin cells (Figure 4). Concentrations of 0.005, 0.025, 0.05 and 0.1 percent 9500 induced 87, 91, 58 and 15 percent relative survival, respectively. S9 mediated metabolism did not significantly alter the cytotoxicity of Corexit 9500 (Figure 4). Concentrations of 0.005, 0.025, 0.05 and 0.1 percent 9500 with S9 fractions induced 83, 83, 51 and 4 percent relative survival, respectively.

Corexit 9527 also induced a concentration-dependent increase in cytotoxicity in humpback cells (Figure 5). Concentrations of 0.005, 0.025, 0.05 and 0.1 percent 9527 induced 78, 80, 71 and 27 percent relative survival, respectively. S9 mediated metabolism resulted in a similar dose response (Figure 5). Concentrations of 0.005, 0.025, 0.05 and 0.1 percent 9527 with S9 fractions induced 89, 81, 75 and 2 percent relative survival, respectively.

Comparison of Corexit 9500 and Corexit 9527 shows there is no significant difference in cytotoxicity between the different dispersant types in humpback skin cells (Figure 6).

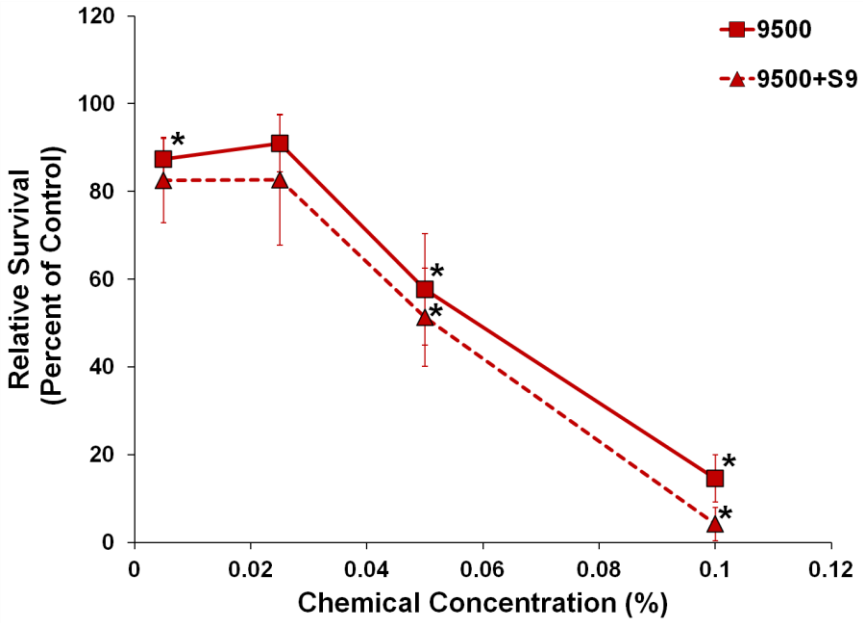

Figure 4. Corexit 9500 is cytotoxic to humpback whale skin cells. This figure shows that Corexit 9500 was cytotoxic to humpback whale skin cells after a 24 h exposure and S9 mediated metabolism did not alter cytotoxicity (measured as cell survival relative to the control). The overall dose-response is highly significant ($p < 0.005$). There was no statistical difference observed between 9500 and 9500+ S9 ($p > 0.05$). Data represent 3 experiments ± the standard error of the mean. *indicates doses that are significantly different from control ($p < 0.05$).

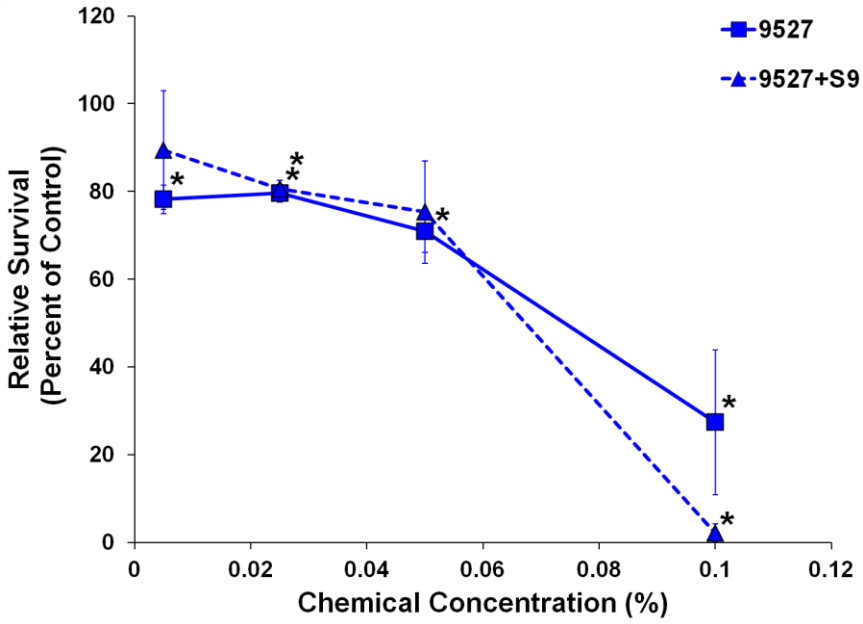

Figure 5. Corexit 9527 is cytotoxic to humpback whale skin cells. This figure shows Corexit 9527 was cytotoxic to humpback whale skin cells in a concentration dependent manner after a 24 h. Cytotoxicity is measured as cell survival relative to the control. There was no statistical difference observed between 9527

and 9527+ S9 ($p > 0.05$). Data represent 3 experiments ± the standard error of the mean. *indicates doses that are statistically significant from control ($p < 0.05$).

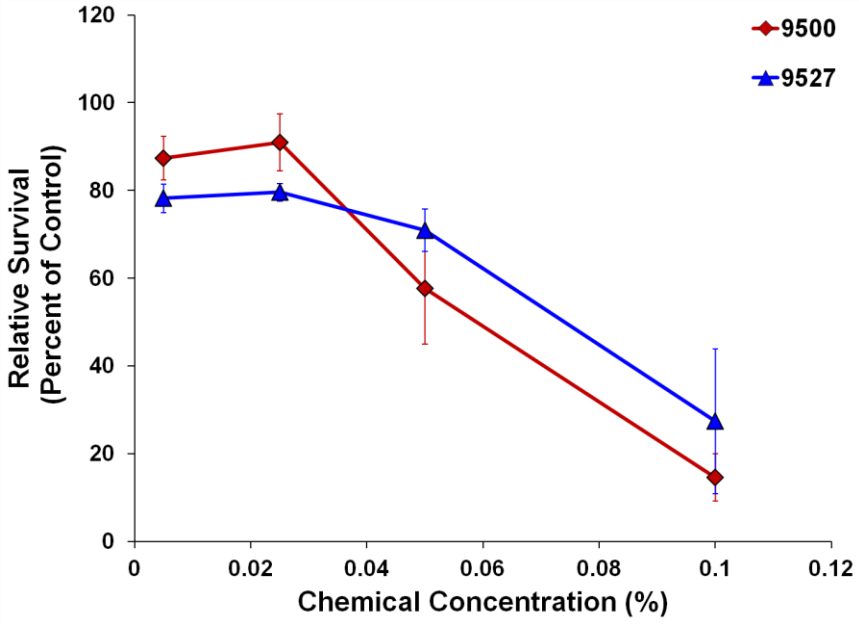

Figure 6. Corexit 9500 and Corexit 9527 induce similar cytotoxic effects in humpback whale skin cells. This figure compares the cytotoxicity of Corexit 9500 and Corexit 9527 after a 24 h exposure. There was no statistically significant difference in the cytotoxicity of Corexit 9500 and Corexit 9527 in humpback skin cells ($p > 0.05$).

Corexit 9500 and 9527 induced similar amounts of cytotoxicity in both sperm whale and humpback whale cells (Figure 7). S9-mediated metabolism did not alter the cytotoxic effect in either cell line. Corexit 9500 did not induce genotoxicity in either cell line; the S9-mediated metabolism did not affect genotoxicity (Figure 8A). Corexit 9527 induced a concentration-dependent increase in genotoxicity in sperm whale cells only, and S9-mediated metabolism increased the genotoxicity slightly (Figure 8B).

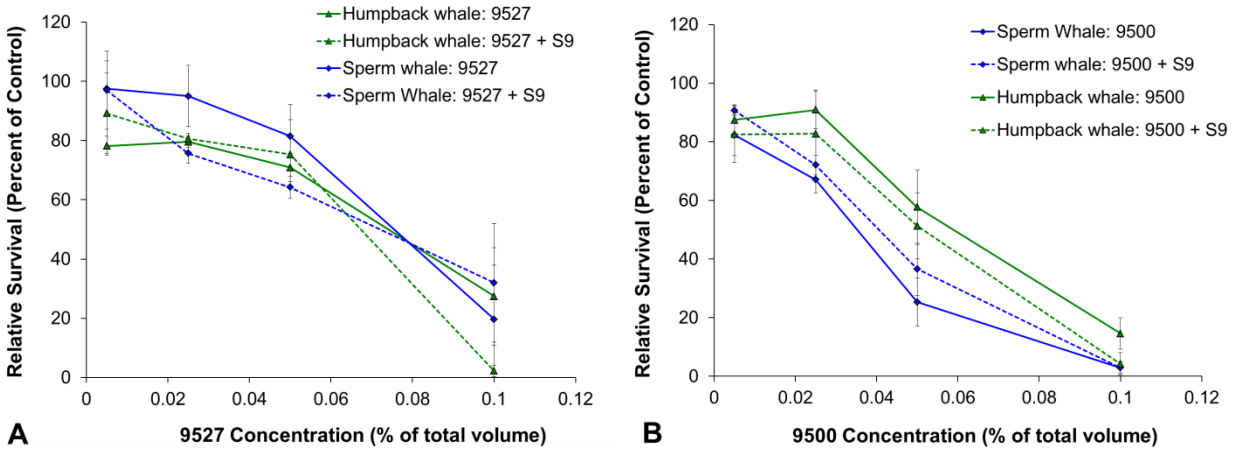

Figure 7. Corexit 9500 and Corexit 9527 induce similar cytotoxic effects in humpback and sperm whale skin cells. This figure compares the cytotoxicity of Corexit 9500 and Corexit 9527 after a 24 h exposure between two whale cell lines. **A)** Corexit 9527. **B)** Corexit 9500. Humpback whale cells and sperm whale cells have a similar cytotoxic response to Corexit 9500 and 9527.

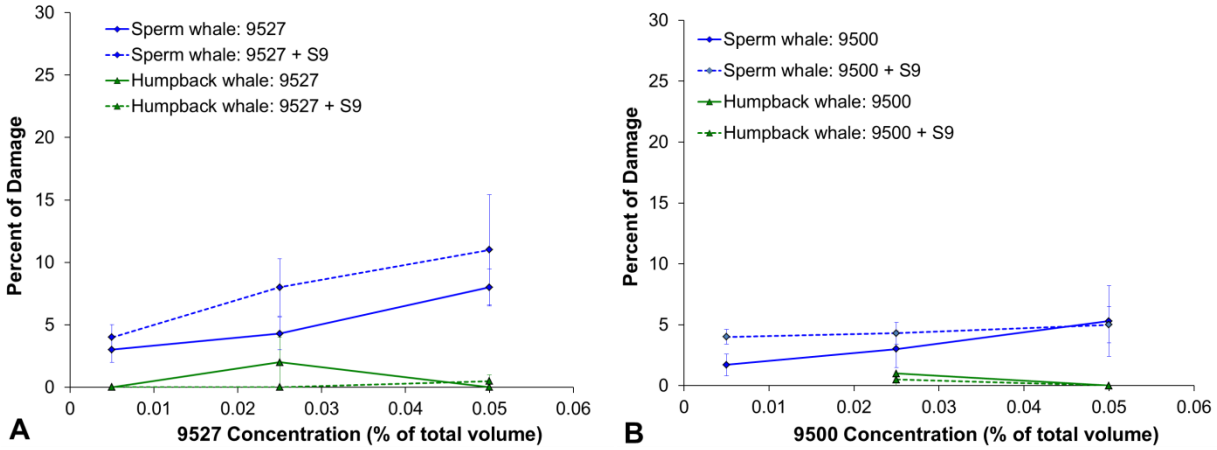

Figure 8. Corexit 9527 is only genotoxic to sperm whale skin cells, Corexit 9500 is not gentotoxic. This figure compares the cytotoxicity of Corexit 9500 and Corexit 9527 after a 24 h exposure between two whale cell lines. Only Corexit 9527 was genotoxic and only to sperm whale cells. **A)** Corexit 9527. **B)** Corexit 9500.

Corexit 9500 and 9527 Clastogenicity in Sperm Whale Skin Cells

Corexit 9500 induced a minimal increase in genotoxicity in sperm whale skin cells (Figure 9). S9 mediated metabolism had no effect on the genotoxicity of Corexit 9500 (Figure 9). Specifically, concentrations of 0.005, 0.025 and 0.05 percent 9500 damaged 1.7, 3 and 5.3 % of

metaphases and induced 2.3, 3 and 5.7 total aberrations per 100 metaphases after subtracting the control levels, respectively. S9 mediated metabolism damaged 3.7, 4 and 4.7 % of metaphases and induced 3.3, 3.3 and 2.7 total aberrations per 100 metaphases after subtracting the control levels, respectively.

By contrast, Corexit 9527 induced a concentration-dependent increase in genotoxicity after 24 h exposure in sperm whale cells (Figure 10). Specifically, concentrations of 0.005, 0.025, 0.05 and 0.1 percent 9527 damaged 3, 4.3, 8 and 10.6 % of metaphases and induced 4, 5, 9.3 and 12.7 total aberrations per 100 metaphases after subtracting the control levels, respectively. S9 mediated metabolism increased this effect damaging chromosomes in 4, 8, 11 and 19.5 % of metaphases and induced 4, 8.7, 14.3 and 25 total aberrations per 100 metaphases after subtracting the control levels, respectively (Figure 10). The spectrum of chromosome aberrations for both compounds consisted of mostly chromatid lesions (Table 1).

Comparisons of Corexit 9500 and 9527 show that 9257 is more genotoxic than 9500 (Figure 11). Corexit 9527 with S9 fractions was the most genotoxic condition, inducing the most total chromosome damage. Corexit 9527 had a higher amount of isochromatid lesions than 9500. Corexit 9500 had a higher amount of dicentric chromosomes and double minutes. The double minutes only occurred in the S9 treated cells. There were few chromatid exchanges in both compounds. Double minutes, dicentrics and chromatid exchanges were not present in any of the controls (Table 1).

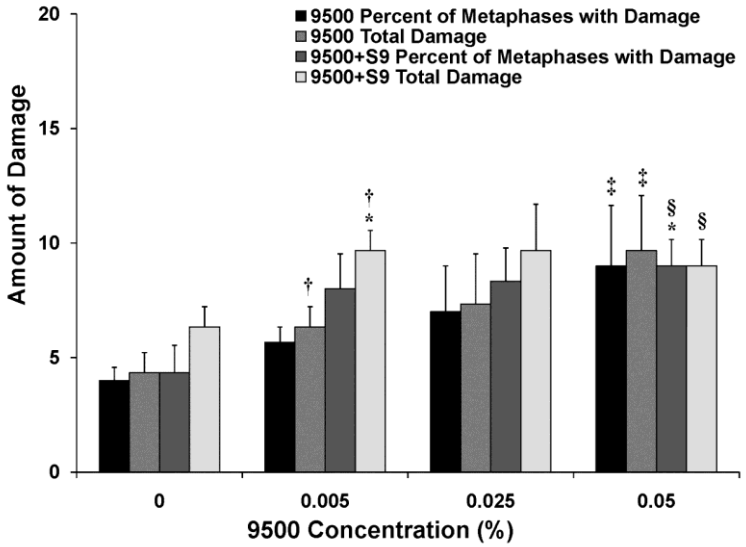

Figure 9. Corexit 9500 is not strongly genotoxic to sperm whale skin cells. This figure shows Corexit 9500 was not substantially and generally not statistically significantly genotoxic to sperm whale skin cells after a 24 h exposure both with and without S9-mediated metabolism. Data are expressed as the average percent of metaphases with damage or total aberrations in 100 metaphases. Data represent 3 experiments ± the standard error of the mean. †Indicates that 9500 is statistically significant from 9500+S9 ($p < 0.05$). *****indicates doses that are significantly different from control ($p < 0.05$). ‡indicates that in one experiment only 68 metaphases could be scored. §indicates that in one experiment only 86 metaphases could be scored.

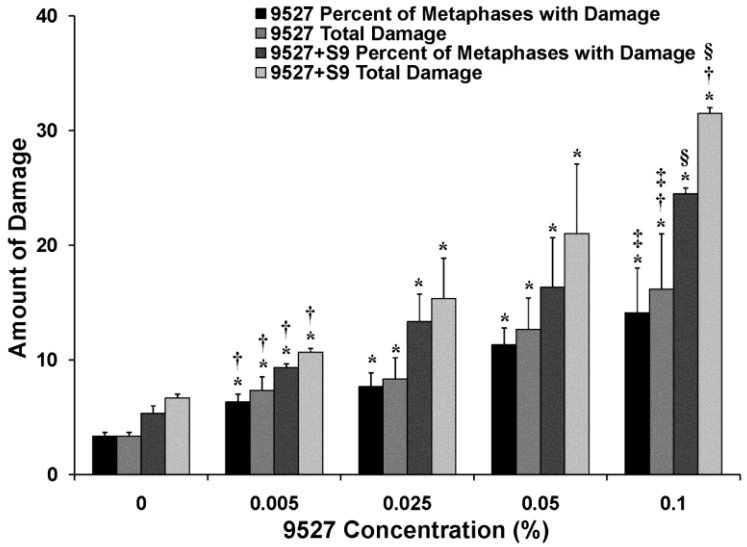

Figure 10. Corexit 9527 is genotoxic to sperm whale skin cells. This figure shows Corexit 9527 induces a concentration-dependent increase in genotoxicity in sperm whale skin cells after a 24 h exposure. S9 mediated metabolism increases genotoxicity. Data are expressed as the average percent of metaphases with damage or total aberrations in 100 metaphases. The effect for S9 increased with concentration ($p = 0.0005$ for interaction based on percent damage and $p = 0.0003$ for interaction based on total damage). The effect for concentration without S9-mediated metabolism was significant for both percent damage ($p = 0.002$) and for total damage ($p = 0.011$). When evaluated in a multivariate regression and at a concentration of 0.05 percent, the effect for S9 was significant for both percent damage and total damage ($p < 0.0001$). Data represent 3 experiments ± the standard error of the mean. 0.1% 9500 with and without S9 was not done for the third experiment because at these concentrations not enough metaphases could be obtained due to cell cycle arrest. *indicates doses that were significantly different from control ($p < 0.05$). †indicates that 9527 was significantly different from 9527+S9 ($p < 0.05$). ‡indicates that in one experiment only 88 could be scored. §indicates that in one experiment only 54 metaphases could be scored respectively.

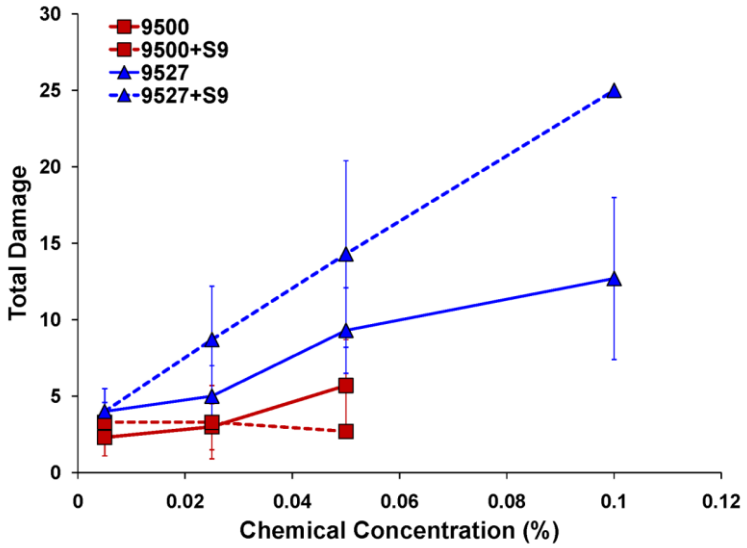

Figure 11. Corexit 9527 induces more total chromosome damage than Corexit 9500. This figure shows the comparison of the total chromosome damage induced by Corexit 9500 and 9527 with and without S9 fractions after a 24 h exposure. Based on a multiple regression model, there was a significant 3-way interaction involving dispersant, concentration and S9 ($p = 0.023$). When evaluated at a concentration of 0.05 percent, Corexit 9527 was significantly more genotoxic than 9500 with S9 fractions ($p < 0.001$) but not without S9 fractions ($p = 0.33$). Data represent the aberrations in 100 metaphases shown in Figures 9 and 10 minus their respective negative control levels.

Table 1. Spectrum of Chromosome Aberrations in Sperm Whale Cells[a]

Concentration	Chromatid lesions	Isochromatid lesions	Chromatid exchanges	Rings	Double minutes	Acentric fragments	Dicentric
Corexit 9500							
0	3	0	0	0	0	0	0
0.005	5	0	1	0	0	0	0
0.025	2	0	0	0	0	0	1
0.05	11	1	0	0	0	0	1
0+S9	1	0	0	0	4	0	1
0.005+S9	6	0	0	0	1	0	1
0.025+S9	5	0	0	0	1	0	0
0.05+S9	8	1	0	0	0	0	0
Corexit 9527							
0	3	0	0	0	0	0	0
0.005	2	6	0	0	0	0	0
0.025	12	0	0	0	0	0	0
0.05	17	0	1	0	0	0	0
0+S9	3	4	0	0	0	0	0
0.005+S9	10	1	0	0	0	0	0
0.025+S9	20	2	0	0	0	0	0
0.05+S9	31	0	0	0	0	0	0

[a]Data are from one representative experiment for each chemical

Corexit 9500 and 9527 Clastogenicity in Humpback Whale Skin Cells

Corexit 9500 did not induce an increase in genotoxicity in humpback whale skin cells (Figure 12). S9 mediated metabolism had no effect on the genotoxicity of Corexit 9500 (Figure 12). Specifically, concentrations of 0.025 and 0.05 percent 9500 damaged 1 and 0 % of metaphases and induced 1.5 and 0 total aberrations after subtracting the control levels, respectively. S9 mediated metabolism damaged 0.5 and 0 % of metaphases and induced 0.5 and 0 % total aberrations after subtracting the control levels, respectively. At the highest concentration there was evidence of cell cycle arrest as in one experiment only 40 and 13 metaphases were observed for 9500 and 9500 with S9, respectively.

Corexit 9527 did not induce genotoxicity after 24 h exposure in humpback whale cells (Figure 13). Specifically, concentrations of 0.005, 0.025, and 0.05 percent 9527 damaged 0, 2, and 0 % of metaphases and induced 0, 2, and 0.3 total aberrations per 100 metaphases after subtracting the control levels, respectively. S9 mediated metabolism had no effect on the genotoxicity of Corexit 9527. Because there was no significant increase in genotoxicity with either compound in humpback cells no detail on aberration type is provided.

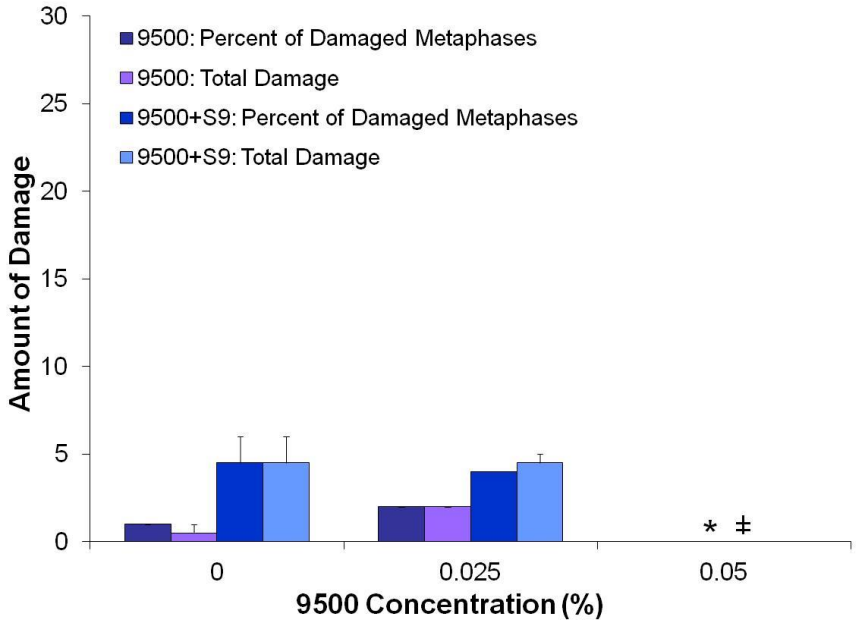

Figure 12. Corexit 9500 is not genotoxic to humpback whale skin cells. This figure shows Corexit 9500 was not genotoxic to humpback whale skin cells after a 24 h exposure both with and without S9-mediated metabolism. Data are expressed as the average percent of metaphases with damage or total aberrations in 100 metaphases. Data represent 2 experiments ± the standard error of the mean. * indicates that only 40 metaphases were observed at this concentration in one experiment. ǂ indicates that only 13 metaphases were observed at this concentration.

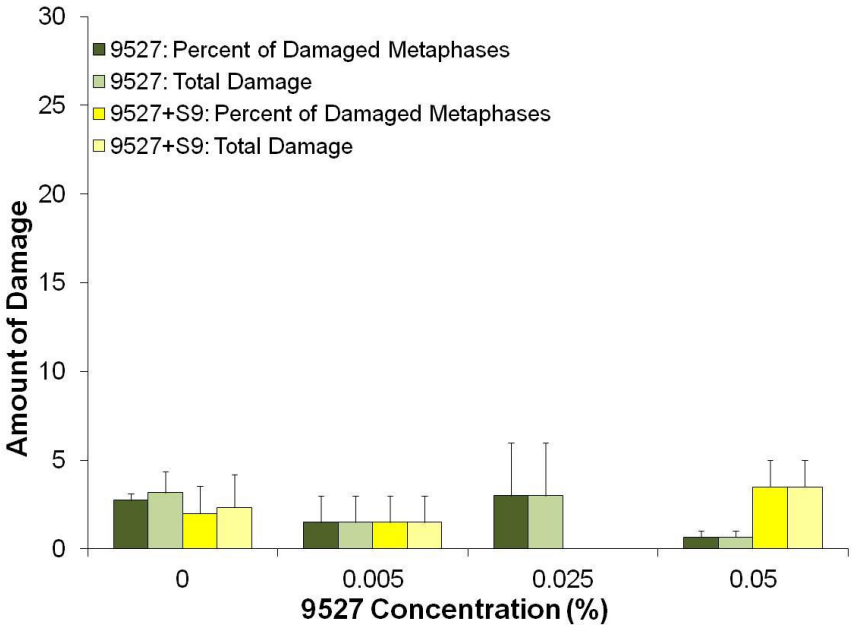

Figure 13. Corexit 9527 is not genotoxic to humpback whale skin cells. This figure shows Corexit 9527 did not induce genotoxicity in humpback whale skin cells after a 24 h exposure. S9 mediated metabolism did not increase genotoxicity. Data are expressed as the average percent of metaphases with damage or total aberrations in 100 metaphases. Data represent 2-3 experiments ± the standard error of the mean.

WAF and CEWAF Cytotoxicity in Sperm Whale Skin Cells

WAF was not cytotoxic to sperm whale skin cells (Figure 14). Concentrations of 1, 5, 10 and 20 percent WAF induced 97.4, 95, 93.5 and 91.5 percent relative survival, respectively. S9 mediated metabolism did not significantly alter the cytotoxicity of WAF. Concentrations of 1, 5, 10 and 20 percent WAF with S9 fractions induced 97.3, 96.3, 95.3, 92 percent relative survival, respectively.

CEWAF induced a concentration-dependent increase in cytotoxicity (Figure 15). Concentrations of 0.5, 1, 5 and 10 percent CEWAF induced 87.8, 88.3, 78.5 and 46.7 percent relative survival, respectively. S9 mediated metabolism resulted in a similar dose response. Concentrations of 0.5, 1, 5 and 10 percent CEWAF with S9 fractions induced 92.3, 91, 84.7 and 22.7 percent relative survival, respectively.

CEWAF is significantly more cytotoxic to sperm whale skin cells than WAF (Figure 16). Specifically, at the treatment concentration of 10%; the cell survival for CEWAF was 50% the amount that it was for WAF.

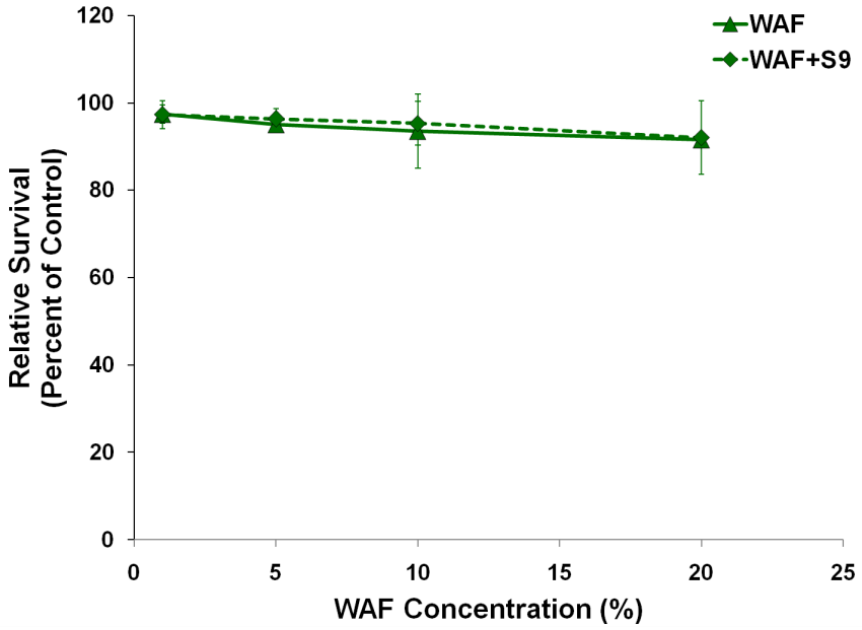

Figure 14. WAF is not cytotoxic to sperm whale skin cells. This figure shows that at concentrations of 0.5-20% WAF was not cytotoxic to sperm whale skin cells following a 24 h exposure. S9 mediated metabolism did not alter the cytotoxicity of WAF. The relative cell survival is calculated as the percent of the control ± S.E. Data represent an average of 4-5 experiments.

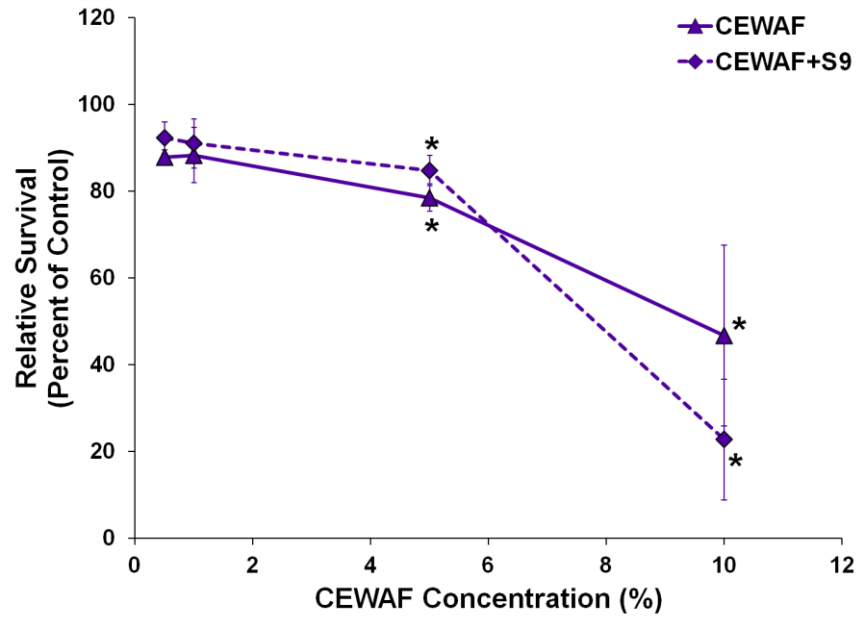

Figure 15. CEWAF is cytotoxic to sperm whale skin cells. This figure shows CEWAF was cytotoxic to sperm whale skin cells following a 24 h exposure. S9 mediated metabolism did not alter the cytotoxicity of CEWAF. The relative cell survival is calculated as the percent of the control ±S.E. *****indicates doses that are statistically significantly different from control ($p < 0.05$). Data represent an average of 3-4 experiments.

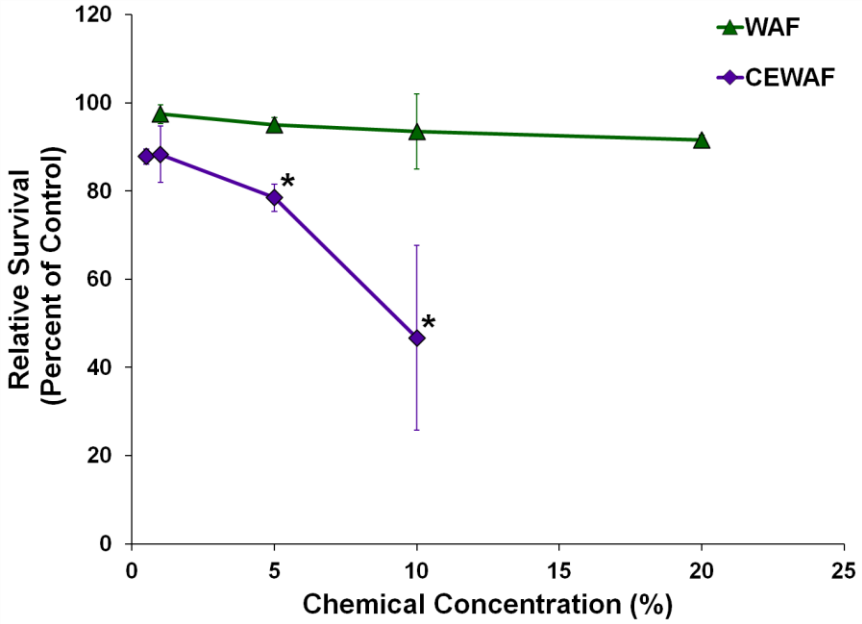

Figure 16. CEWAF is more cytotoxic than WAF to sperm whale skin cells. This figure shows the cytotoxic response comparison of sperm whale skin cells after 24 h exposure to WAF and CEWAF. These cells are more sensitive to CEWAF than WAF. *indicates concentrations that were statistically significantly different from each other ($p < 0.05$). Data represent an average of 3 experiments.

WAF and CEWAF Cytotoxicity in Humpback Whale Skin Cells

WAF was not cytotoxic to humpback whale skin cells (Figure 17). Concentrations of 1, 5, 10 and 20 percent WAF induced 93, 92, 90 and 84 percent relative survival, respectively. S9 mediated metabolism did not significantly alter the cytotoxicity of WAF. Concentrations of 1, 5, 10 and 20 percent WAF with S9 fractions induced 92, 99, 89, and 90 percent relative survival, respectively.

CEWAF induced a concentration-dependent increase in cytotoxicity (Figure 18). Concentrations of 0.5, 1, 5 and 10 percent CEWAF induced 99, 96, 86 and 66 percent relative survival, respectively. S9 mediated metabolism increased toxicity at the highest concentration. Concentrations of 0.5, 1, 5 and 10 percent CEWAF with S9 fractions induced 99, 95, 80 and 31 percent relative survival, respectively.

CEWAF is not significantly more cytotoxic to sperm whale skin cells than WAF (Figure 19). However, S9 mediated metabolism of CEWAF was significantly more toxic than S9 mediated metabolism of WAF.

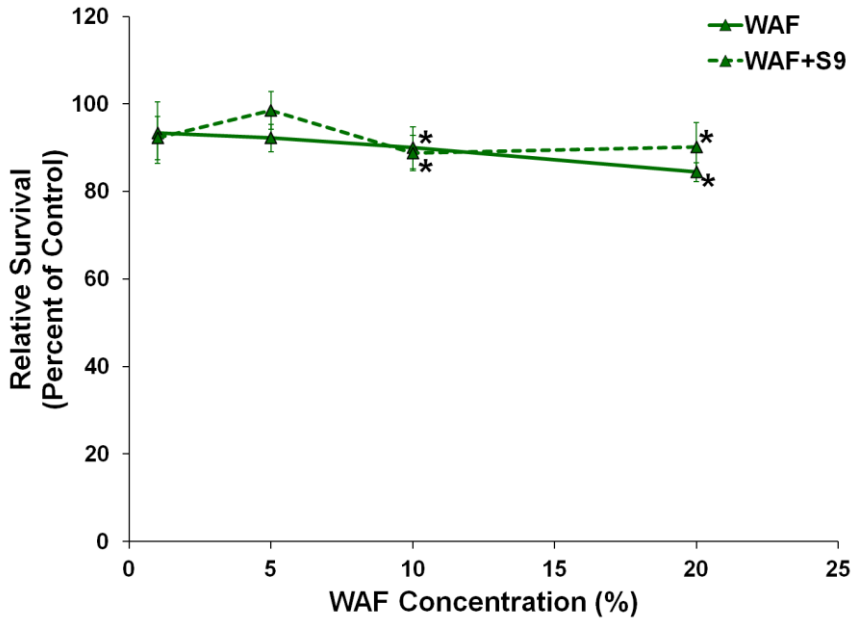

Figure 17. WAF is slightly cytotoxic to humpback whale skin cells. This figure shows that at concentrations of 0.5-5% WAF was not cytotoxic to humpback whale skin cells following a 24 h exposure, but the higher concentrations of 10 and 20% were more cytotoxic compared to controls both with and without S9 metabolism. S9 mediated metabolism, however, did not alter the cytotoxicity of WAF. The relative cell survival is calculated as the percent of the control ±S.E. Data represent an average of 4-5 experiments. *statistically significantly different from control ($p < 0.05$).

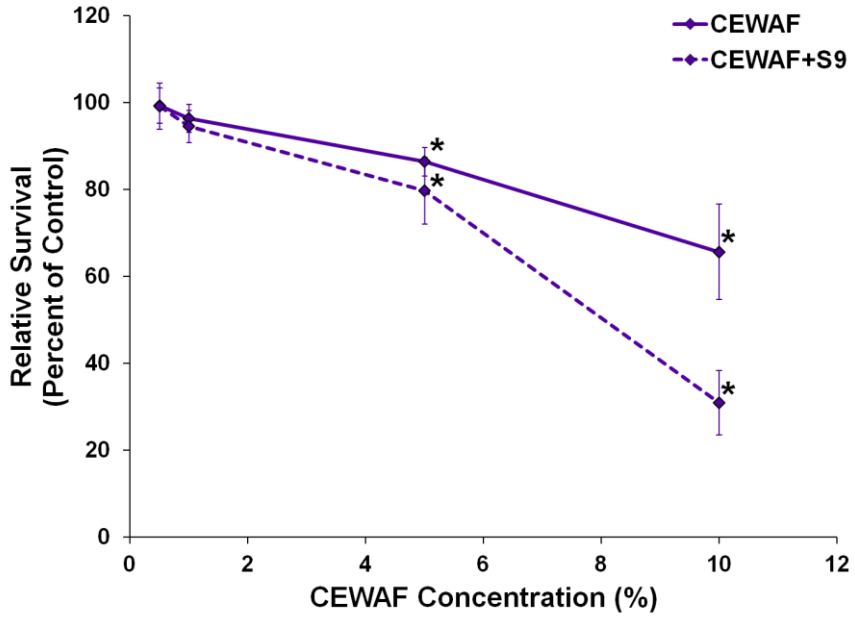

Figure 18. CEWAF is cytotoxic to humpback whale skin cells. This figure shows CEWAF was cytotoxic to humpback whale skin cells following a 24 h exposure. S9 mediated metabolism was only significant at

the highest concentration of CEWAF ($p < 0.05$). The relative cell survival is calculated as the percent of the control ±S.E. ***** indicates concentrations that are statistically significantly different from control ($p < 0.05$). Data represent an average of 3-4 experiments.

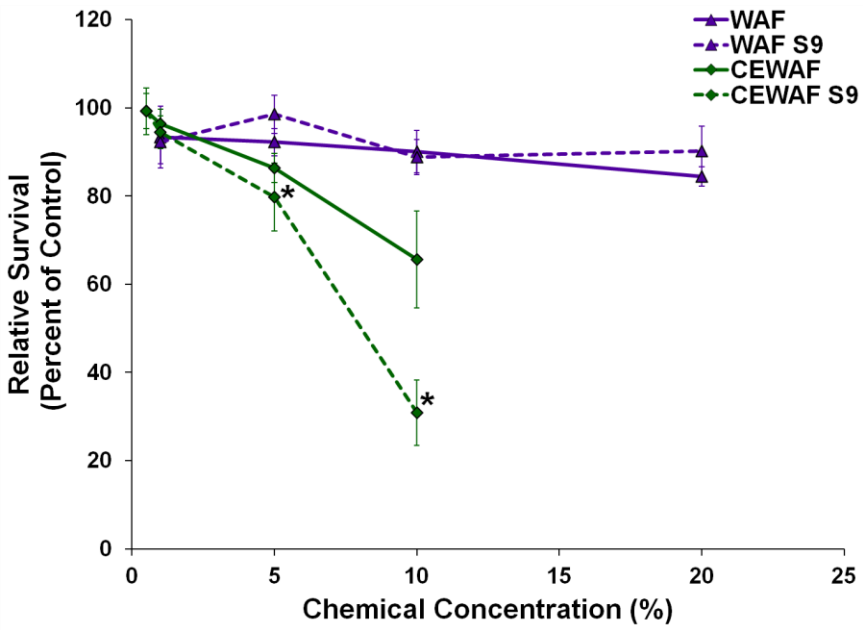

Figure 19. CEWAF is more cytotoxic than WAF to humpback whale skin cells. This figure shows the cytotoxic comparison of humpback whale skin cells after 24 h exposure to WAF and CEWAF with and without S9 metabolism. When S9-mediated metabolism occurs, these cells are more sensitive to CEWAF than WAF. *indicates S9-treated concentrations that were statistically significantly different from non-S9-treated ($p < 0.05$). Data represent an average of 4-5 experiments.

WAF and CEWAF Clastogenicity in Sperm Whale Skin Cells

Following 24 h exposure to WAF sperm whale skin cells exhibited a concentration-dependent increase in genotoxicity (Figure 20). Specifically, concentrations of 1, 5 and 10 % WAF induced damage in 0.7, 3, 4 % of metaphases and induced damage in 1.3, 3.3, 5.7 total aberrations per 100 metaphases, respectively, after accounting for background levels by subtracting out the control. Genotoxicity was increased by S9 mediated metabolism. S9 induced damage in 2.7, 4.3, 8.3 % of metaphases and damaged 4, 4.7, 10.3 total aberrations per 100 metaphases, respectively, after accounting for background levels.

CEWAF induced a concentration-dependent increase in genotoxicity after 24 h exposure in sperm whale skin cells (Figure 21). Specifically, concentrations of 0.5, 1 and 5 % CEWAF induced damage in 2, 4, 4.3 % of metaphases and induced damage in 2, 4, 4.7 total aberrations per 100 metaphases, respectively, after accounting for background levels by subtracting out the control. S9 mediated metabolism increased genotoxicity. Specifically, CEWAF+S9 induced damage in 5.3, 9.7, 14 % of metaphases and damaged 6.7, 10.7, 17.3 total aberrations per 100 metaphases, respectively, after accounting for background levels. The percent of damaged metaphases tripled at the highest dose when phase 1 metabolism was induced outside of the cell.

Comparison of the total damage induced by each compound shows that CEWAF is slightly more genotoxic than WAF (Figure 22). S9 mediated metabolism not only increased the genotoxicity of each compound but also increased the difference in genotoxicity between CEWAF and WAF. CEWAF + S9 is more genotoxic than WAF+S9. Chromatid lesions were the most frequent aberration induced by WAF and CEWAF (Table 2).

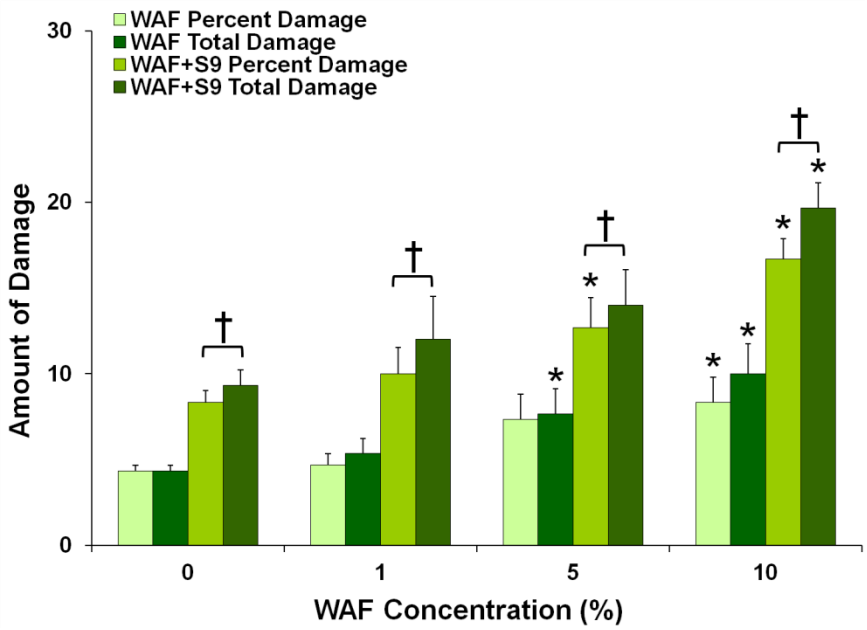

Figure 20. WAF is genotoxic to sperm whale skin cells. This figure shows after 24 h exposure WAF induced a concentration-dependent increase in genotoxicity in sperm whale cells. S9 mediated metabolism slightly increases genotoxicity. Data are expressed as the average percent of metaphases with damage or total aberrations in 100 metaphases. *****indicates doses that are statistically significantly different from control ($p < 0.05$). †Indicates that WAF is statistically significant from WAF+S9 ($p < 0.05$). Data represent an average of 3 experiments.

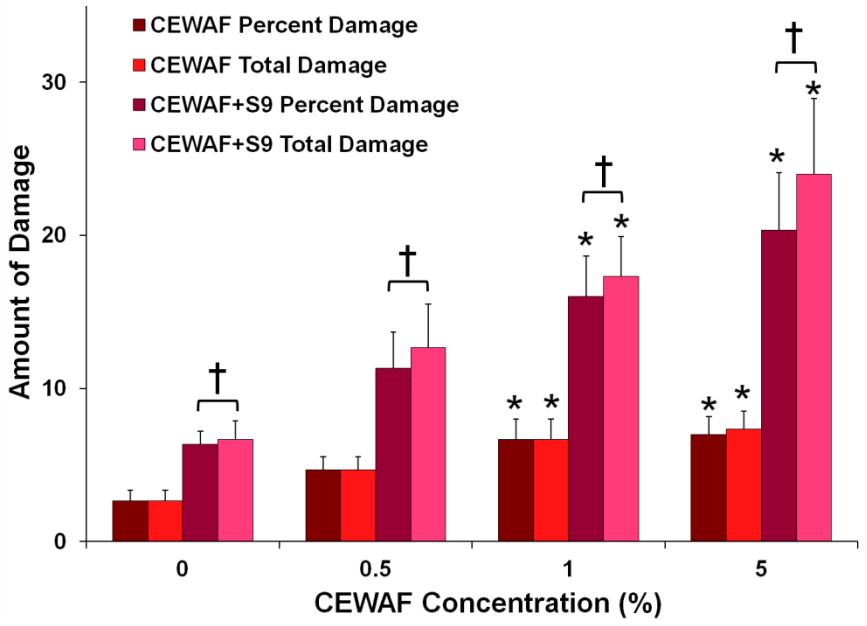

Figure 21. CEWAF is genotoxic to sperm whale skin cells. This figure shows that CEWAF was genotoxic to sperm whale cells in a concentration-dependent manner. S9 mediated metabolism increased the amount of damage caused by CEWAF. Data are expressed as the average percent of metaphases with damage or total aberrations in 100 metaphases. *****indicates doses that are statistically significantly different from control ($p < 0.05$). †Indicates that CEWAF is statistically significant from CEWAF+S9 ($p < 0.05$). Data represent an average of 3 experiments.

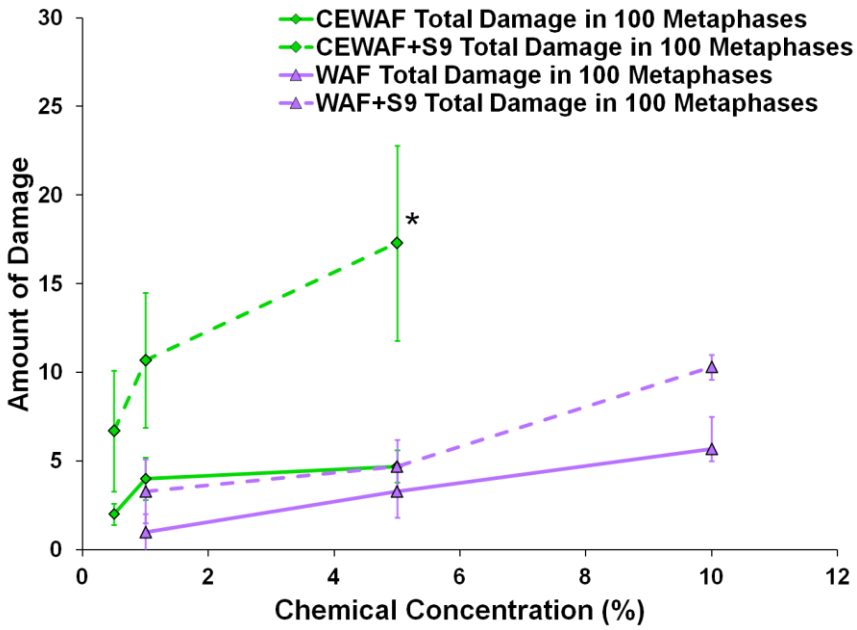

Figure 22. CEWAF induces more total chromosome damage than WAF. This figure shows the comparison of total damage induced by WAF, WAF+S9, CEWAF and CEWAF+S9. CEWAF+S9 was the most genotoxic of the four conditions to sperm whale cells (*$p < 0.05$). Data are expressed as the total aberrations in 100 metaphases. Controls were subtracted from each value. Data represent an average of 3 experiments.

Table 2. Spectrum of Chromosome Aberrations in Sperm Whale Cells[a]

	Chromatid lesions	Isochromatid lesion	Chromatid exchange	Ring	Double minute	Acentric fragment	Dicentric
WAF							
0	4	1	0	0	0	0	0
1	4	0	0	0	0	0	1
5	8	0	0	0	0	0	0
10	8	1	0	0	0	0	1
0+S9	9	0	0	0	0	0	0
1+S9	7	0	0	0	0	0	0
5+S9	10	0	0	0	0	1	0
10+S9	19	0	0	0	0	1	0
CEWAF							
0	2	0	0	0	0	0	0
0.5	2	1	0	0	0	0	0
1	6	2	0	0	0	0	0
5	7	0	0	0	0	1	0
0+S9	6	0	0	0	0	0	0
0.5+S9	14	1	0	0	0	0	0
1+S9	16	0	1	0	0	0	0
5+S9	16	0	0	0	0	0	0

[a]Data are from one representative experiment for each chemical

WAF and CEWAF Clastogenicity in Humpback Whale Skin Cells

WAF was not genotoxic to humpback whale skin cells after a 24 h treatment (Figure 23). Specifically, concentrations of 5 and 10 % WAF induced damage in 0 and 0 % of metaphases, respectively, after accounting for background levels by subtracting out the control. Genotoxicity was increased slightly by S9 mediated metabolism. S9 induced damage in 4 and 6% of metaphases, respectively, after subtracting the control levels.

CEWAF did not induce an increase in genotoxicity after 24 h exposure in humpback whale skin cells (Figure 24). Specifically, concentrations of 0.5, 1 and 5 % CEWAF induced damage in 2, 1.8, 2.1 % of metaphases and induced damage in 2.3, 1.8, 2.1 total aberrations per 100 metaphases, respectively, after subtracting the control levels. S9 mediated metabolism increased genotoxicity, particularly at the highest concentration. Specifically, concentration of 0.5, 1, and 5% CEWAF+S9 induced damage in 3.1, 3, and 9.1 % of metaphases and damaged 4, 3.3, 11 total aberrations per 100 metaphases after subtracting the control levels, respectively.

Comparison of the total damage induced by each compound shows that CEWAF is more genotoxic than WAF (Figure 25). S9 mediated metabolism not only increased the genotoxicity of each compound but also increased the difference in genotoxicity between the S9-mediated metabolism of CEWAF and WAF. Chromatid lesions were the most frequent aberration induced by WAF and CEWAF (Table 3).

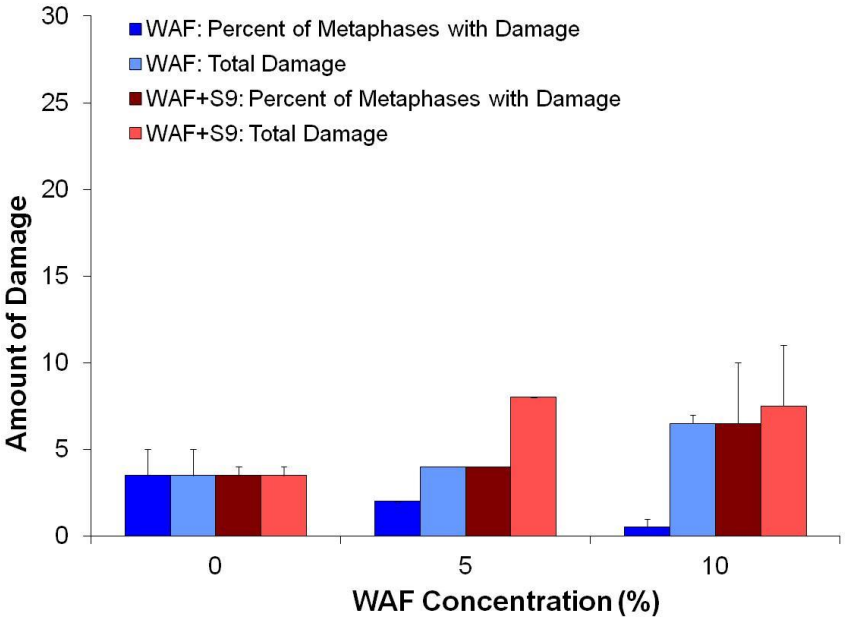

Figure 23. WAF is not genotoxic to humpback whale skin cells. This figure shows after 24 h exposure WAF did not induce genotoxicity in sperm whale cells. S9 mediated metabolism did not significantly increases genotoxicity. Data are expressed as the average percent of metaphases with damage or total aberrations in 100 metaphases. Data represent an average of 2 experiments. No values were statistically significant.

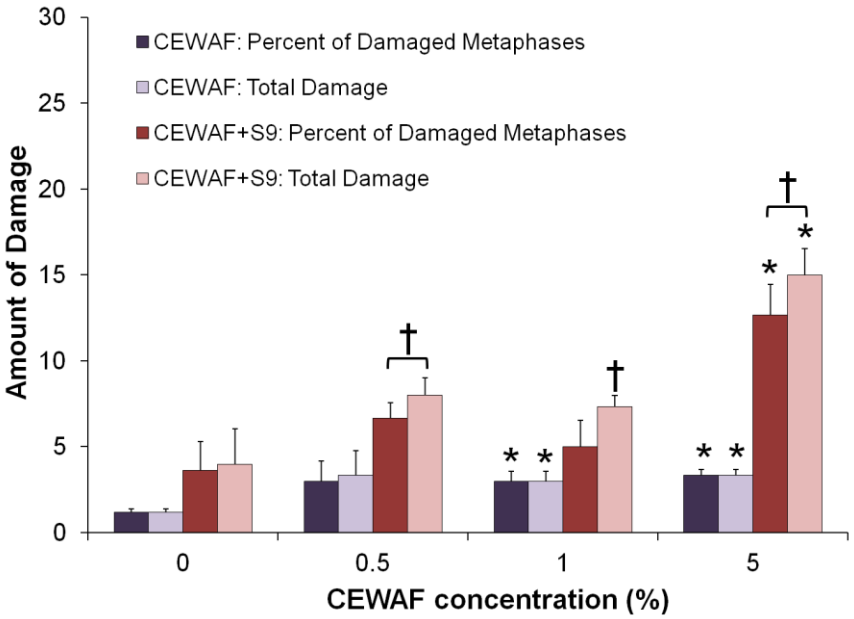

Figure 24. S9-mediated metabolism of CEWAF is genotoxic to humpback whale skin cells. This figure shows that CEWAF was not genotoxic to humpback whale cells after 24 h of exposure. S9 mediated metabolism increased the amount of damage caused by CEWAF. Data are expressed as the average percent of metaphases with damage or total aberrations in 100 metaphases. *****indicates doses that are

statistically significantly different from control ($p < 0.05$). †Indicates that CEWAF is statistically significant from CEWAF+S9 ($p < 0.05$). Data represent an average of 3 experiments.

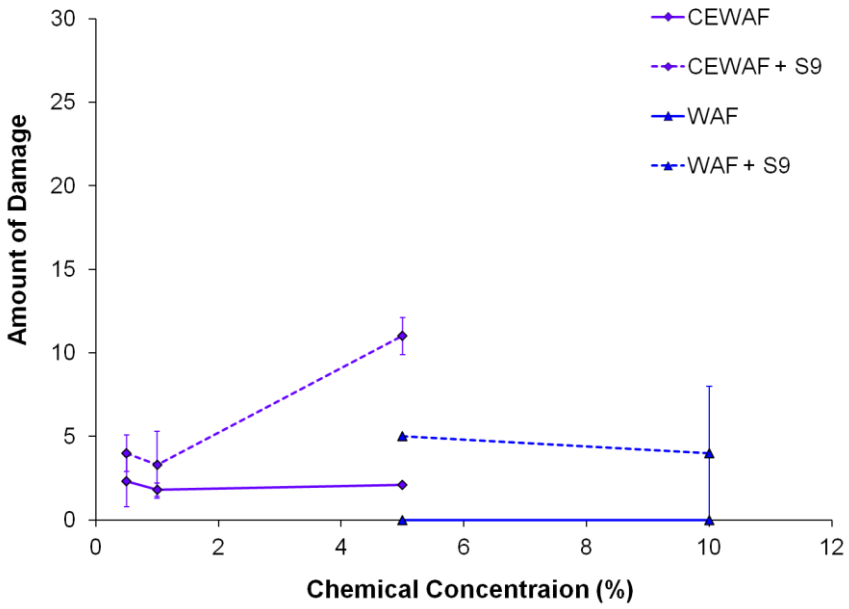

Figure 25. CEWAF induces more total chromosome damage than WAF. This figure shows the comparison of total damage induced by WAF, WAF+S9, CEWAF and CEWAF+S9. CEWAF+S9 was the most genotoxic of the four conditions to humpback whale cells. Data are expressed as the total aberrations in 100 metaphases. Controls were subtracted out. Data represent an average of 2-3 experiments.

Table 3. Spectrum of Chromosome Aberrations in Humpback Whale Cells[a]

	Chromatid lesions	Isochromatid lesion	Chromatid exchange	Ring	Double minute	Acentric fragment	Dicentric
WAF							
0	2	0	0	0	0	0	0
5*	1	0	0	0	0	0	0
10	1	0	0	0	0	0	0
0+S9	3	0	0	0	0	0	0
5+S9‡	4	0	0	0	0	0	0
10+S9	9	1	1	0	0	0	0
CEWAF							
0	1	0	0	0	0	0	0
0.5	4	1	0	0	1	0	0
1	3	0	0	0	0	0	0
5	1	1	0	0	0	0	0
0+S9	3	0	0	0	0	0	0
0.5+S9	5	1	0	0	1	0	0
1+S9	6	0	0	0	0	0	0
5+S9	14	0	0	0	0	0	2

[a]Data shown are from one representative experiment for each chemical; *only 27 metaphases were examined; ‡ only 50 metaphases were examined.

Chemical Analysis of WAF and CEWAF treatments

PAH analysis was performed on the stock solution for both WAF and CEWAF preparations on each day of use. We considered levels of PAHs of significance for Alaskan oil based on previous studies (Incardona et al, 2009; Hatland et al, 2010). Table 4 lists the specific PAHs considered and the average levels found in the WAF and CEWAF preparations. Levels of PAHs from CEWAF were forty times higher than WAF. We analyzed a representative dilution series of the CEWAF preparations to ensure that our treatment conditions were representative of 1, 5, and 10% of the stock solution. (Figure 26). Comparing the values in the graphs to the total levels seen in Table 4, it is clear that treatment conditions accurately represent the given percentages. Specifically, while the average levels of PAHs in the CEWAF stock solution were 39,218,129 ng/L, the 10% dilution was 3,082,645; the 5% dilution was 1,599,318; and the 1% dilution was 355,092.

Using this data, we can better compare the results from the cell culture analysis. WAF and CEWAF were not cytotoxic at similar low total PAH levels but there was a concentration-dependent increase in cytotoxicity with higher PAH levels in both sperm whale and humpback whale skin cells (Figure 27,28). (Higher concentrations of WAF could not be achieved without adversely affecting the level of media necessary to maintain healthy cells). In addition, we can compare between species. The relatively low levels of total PAHs measured in WAF are not cytotoxic to sperm whale and humpback whale cells (Figure 29). Higher levels of total PAHs from the CEWAF induce a concentration-dependent increase in cytotoxicity in both humpback and sperm whale cells; S9 mediated metabolism of the CEWAF increased the cytotoxic effect (Figure 30). Sperm whale cells are slightly more sensitive to higher PAH levels than humpback whale cells.

Similarly, the genotoxic effect of WAF and CEWAF can be analyzed using the measured PAH levels. The lower levels of total PAHs found in WAF are not genotoxic to either sperm

whale or humpback whale cells. S9 mediated metabolism cause a slight increase in damage but not significant (Figure 31). The higher levels of PAHs found in CEWAF alone do not induce a concentration dependent increase in the percent of metaphases damaged; however, S9-mediated metabolism does increase the genotoxic effect of the total PAHs (Figure 32). PAHs induced more damage in the sperm whale cells than humpback whale cells.

Table 4. Levels of PAHs found in oil preparations.

Compound	WAF Concentrations ng/L	CEWAF Concentrations Ng/L
1,4,6,7-Tetramethylnaphthalene	7305 ± 615	309857 ± 29277
1-Methylnaphthalene	65900 ± 4100	2981429 ± 253409
1-Methylphenanthrene	10730 ± 1970	475714 ± 42110
2,3,5-Trimethylnaphthalene	20250 ± 3250	1140000 ± 106077
2,3,6-Trimethylnaphthalene	28150 ± 4250	1592857 ± 142590
2,6-Dimethylnaphthalene	26600 ± 5600	2015714 ± 161759
2-Methylanthracene	1006 ± 324	12791 ± 1784
2-Methylnaphthalene	69600 ± 7400	3915714 ± 332636
Acenaphthene	1345 ± 155	42243 ± 4007
Acenaphthylene	613 ± 89	5669 ± 781
Anthracene	609 ± 9	14414 ± 1292
Benz[a]anthracene	547 ± 141.5	13754 ± 1691
Benzo[a]pyrene	720 ± 96	13336 ± 1218
Benzo[b]fluoranthene	495 ± 83	11806 ± 1203
Benzo[e]pyrene	798 ± 26	27529 ± 2929
Benzo[j,k]fluoranthenes	535 ± 85.5	10531 ± 1078
Biphenyl	12000 ± 1100	503714 ± 39511
C1-Benzo[a]anthracenes/Chrysenes	5935 ± 945	224714 ± 21089
C1-Fluoranthenes/Pyrenes	15050 ± 1350	470286 ± 42803
C2 Phenanthrenes/Anthracenes	45400 ± 4800	1875714 ± 168634
C2-Benzo[a]anthracenes/Chrysenes	6740 ± 1330	239571 ± 25775
C2-Biphenyls	32800 ± 200	450000 ± 39019
C2-Dibenzothiophenes	49800 ± 9000	1870000 ± 161629
C2-Fluoranthenes/Pyrenes	23600 ± 4700	747857 ± 64394
C2-Fluorenes	26850 ± 4850	1039429 ± 86076
C3-Dibenzothiophenes	42050 ± 9250	1417143 ± 110899
C3-Fluoranthenes/Pyrenes	11545 ± 1855	380429 ± 31620
C3-Fluorenes	32200 ± 3700	1187143 ± 73799
C3-Naphthalenes	114500 ± 12500	5885714 ± 519839
C3-Phenanthrenes/Anthracenes	36600 ± 3600	1345714 ± 113155
C4-Dibenzothiophenes	24700 ± 3400	669429 ± 36708
C4-Naphthalenes	62300 ± 4200	2478571 ± 232394
C4-Phenanthrenes/Anthracenes	69300 ± 8300	1891429 ± 166239
Chrysene	2450 ± 790	111114 ± 10845
Dibenz[a,h]anthracene	243 ± 11	6956 ± 556
Dibenzothiophene	11685 ± 2015	522000 ± 43224
Fluoranthene	955 ± 3.5	11327 ± 1200
Fluorene	5260 ± 820	227571 ± 18178
Indeno[1,2,3-cd]pyrene	632 ± 53	6059 ± 580
Naphthalene	100500 ± 500	2288571 ± 187812
Perylene	743 ± 94.5	14314 ± 1392
Phenanthrene	16900 ± 3000	740714 ± 64736
Pyrene	1079 ± 161	29286 ± 2435
Total	987018 ± 108952	39218129 ± 3287878

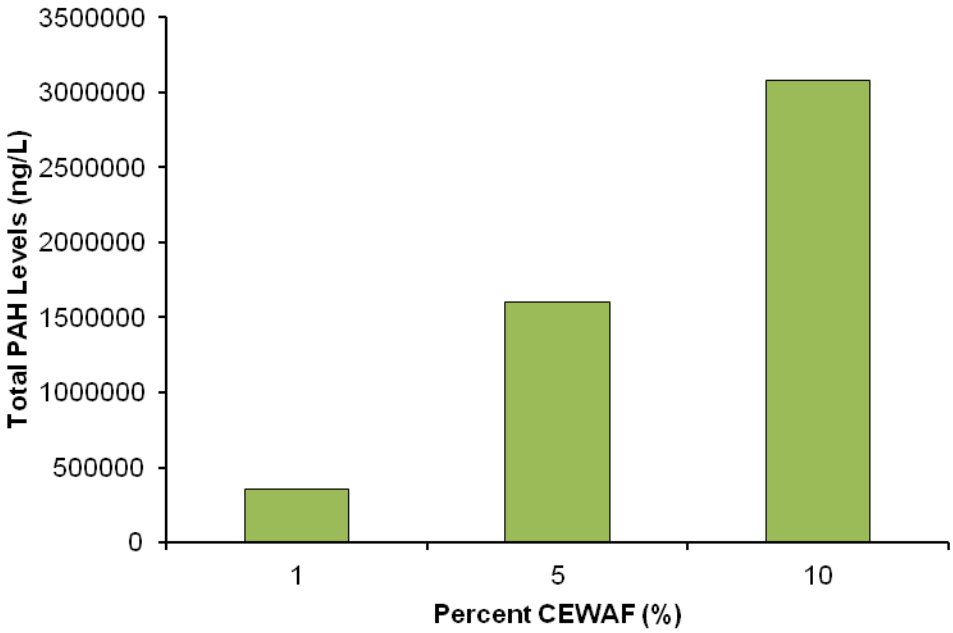

Figure 26. Total PAH present in CEWAF dilutions. This figure shows that the PAHs in each dilution closely represent the given percentage of the total PAH levels used in cell culture treatments. Total level of PAHs in the stock CEWAF was 39,218,129.

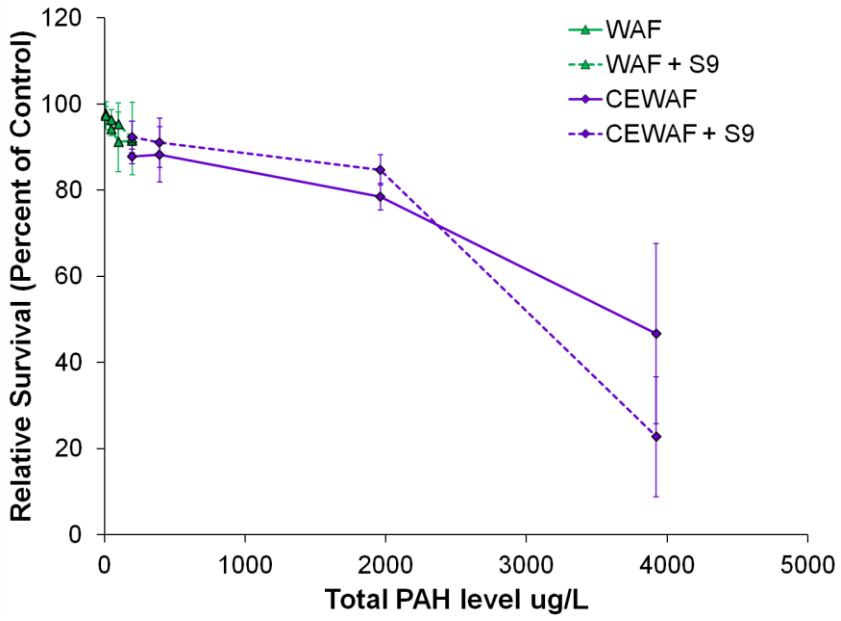

Figure 27. WAF and CEWAF cytotoxicity based on total PAH treatment levels in sperm whale cells. This figure shows that WAF and CEWAF are not cytotoxic and low PAH levels but that higher PAH levels induce a concentration dependent increase in cytotoxicity. Data represent 3-5 experiments ± standard error of the mean.

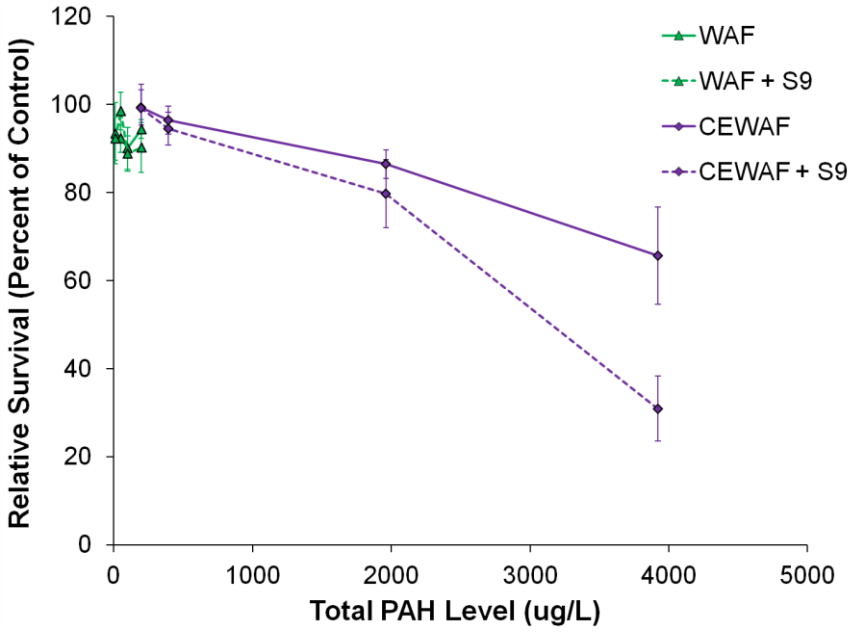

Figure 28. WAF and CEWAF cytotoxicity based on total PAH treatment levels in humpback whale cells. This figure shows that WAF and CEWAF are not cytotoxic and low PAH levels but that higher PAH levels induce a concentration dependent increase in cytotoxicity. Data represent 3-5 experiments ± standard error of the mean.

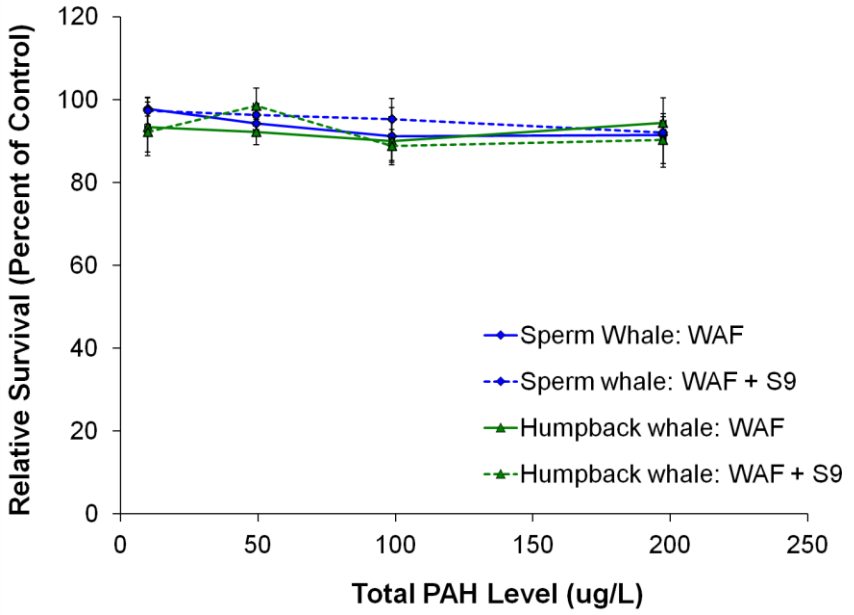

Figure 29. Levels of PAHs found in WAF are not cytotoxic to humpback whale and sperm whale cells. This figure shows that low levels of total PAHs found in WAF are not cytotoxic to humpback and sperm whale cells. Data represent 3-5 experiments ± standard error of the mean.

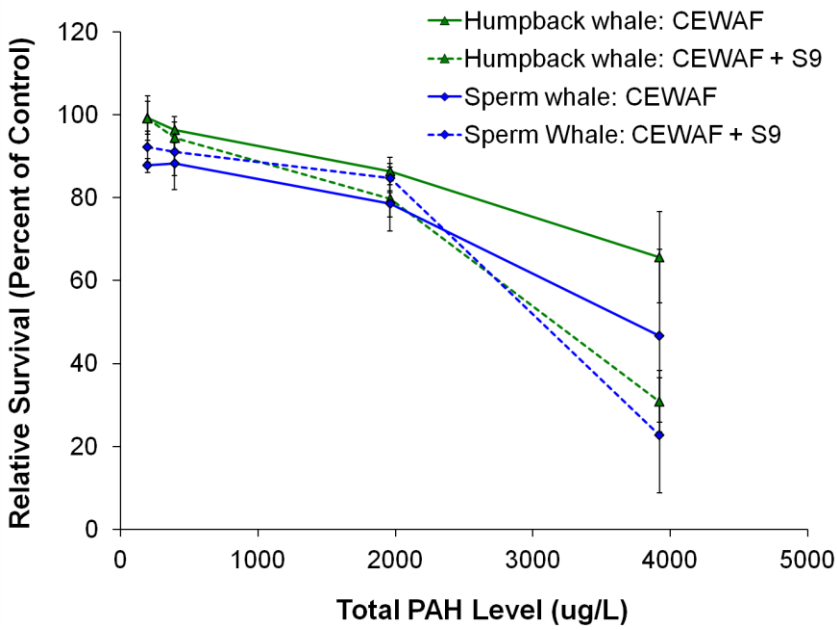

Figure 30. Humpback whale cells and sperm whale cells respond similarly to high PAH levels found in CEWAF. This figure shows that both humpback and sperm whale cells have a similar cytotoxic response to total PAH levels. S9-mediated metabolism increased the cytotoxicity in both cell lines. Data represent 3-5 experiments ± standard error of the mean.

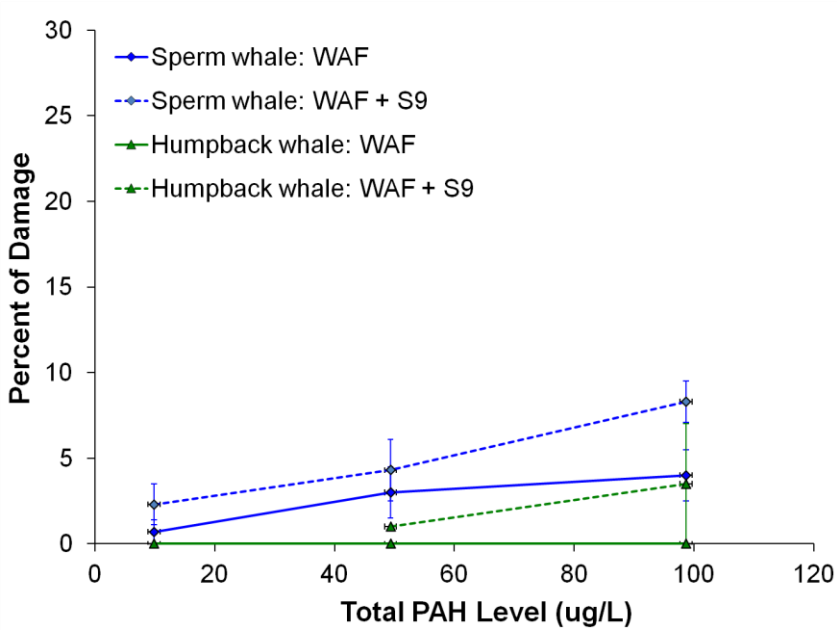

Figure 31. Levels of PAHs found in WAF are not genotoxic to humpback whale and sperm whale cells. This figure shows that low levels of total PAHs found in WAF are not genotoxic to humpback and sperm whale cells, after subtracting the control levels. Data are expressed as the average percent of metaphases with damage. Data represent 2-3 experiments ± standard error of the mean.

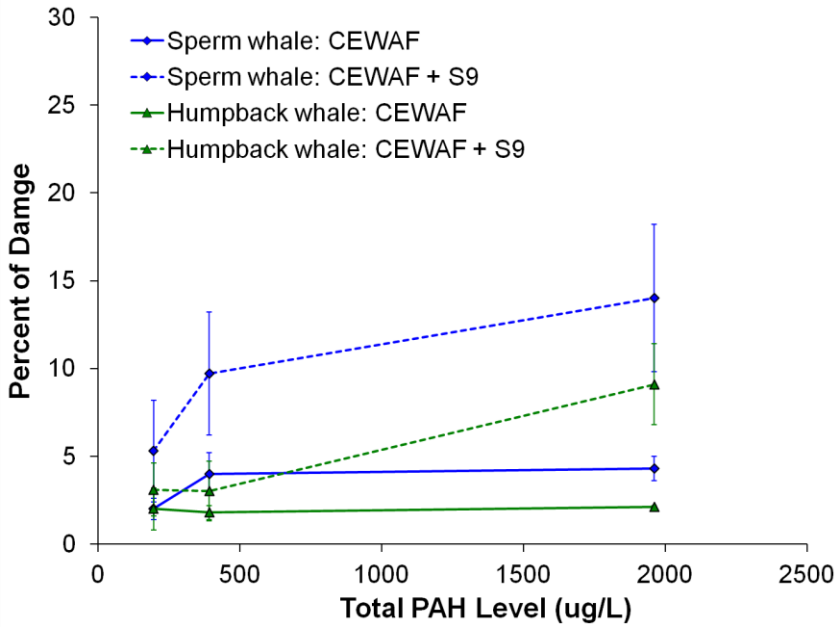

Figure 32. Levels of PAHs found in CEWAF are genotoxic to humpback whale and sperm whale cells. This figure shows that higher levels of total PAHs found in CEWAF are not genootoxic to humpback and sperm whale cells but S9 mediated metabolism induces a concentration-dependent increase in the percent of damaged metaphases, after subtracting the control levels. Data are expressed as the average percent of metaphases with damage. Data represent 2-3 experiments ± standard error of the mean.

Environmental Context for the Doses Used

Determining a “dose” for an oil spill, dispersed oil and dispersants applied is a serious challenge and one made more complex for whales, because of their time spend at the surface. Thus, both water levels and air levels need to be considered. There is no known accurate method to measure the amount of whale exposure to dispersants, oil or dispersed oil in the Gulf of Mexico Deepwater Horizon oil crisis. There likely will not be in future spills either. The reason for this discrepancy is because the whales spend significant amounts of time logging at the surface. Hence, a measured water concentration can be relatively meaningless in exposure scenarios. For example, if oil is on the surface, which it often is in an oil spill, and a whale surfaces into the oil directly, which they have been observed to do, then the exposure to oil becomes 100%, which is way above the levels we used. In contrast, other whales may not surface in oil and have no

exposure. While others may encounter water admixed with oil or something in the range of that measured in the water after a certain amount of equilibrium is reached.

A similar conundrum exists for dispersants. In the Gulf oil spill a final ratio of about 1:63 dispersant to oil was used, i.e. a final concentration of about 1.6% (calculated based on a total applied dispersant amount of approximately 8,000,000 L, reported in Kujawinski et al., 2011 and a total released crude oil amount of 500,000,000 L of crude oil data from Crone and Tolstoy as cited in Joung and Shiller, 2013). Corexit dispersants were sprayed aerially and injected at depth. While some whales may have avoided having any exposure, given the locations of the oil and the spraying and the whales, it is highly likely some whales were indeed directly exposed to dispersants. Because of their well-established logging and deep-diving behaviors, the spectrum of dispersant concentrations the Gulf whales could have encountered would have ranged from very high (i.e. 100%) if the whale was directly exposed to the dispersant spray or stream as it entered the Gulf; to moderate (i.e. 1–50%) if the whale was exposed as the dispersant became mixed with oil and water; to something much lower (i.e. <0.1%) if the whale was exposed after the dispersant mixture dispersed through the water column or food sources. Given these scenarios and the final ratio of dispersant applied, our doses certainly seem plausible.

There will undoubtedly be a shift in discussion from whether or not dispersants and dispersed oil are toxic to a discussion about what a safe exposure level is. It will be important to bear in mind the life history of an animal and that averaged water concentrations or levels after equilibrium do not necessarily tell the whole story. Similarly, it is important to remember that when considering how to extrapolate to a “safe” dose, that for a genotoxic agent, by rule, there is no threshold dose, one extrapolates the curve back to zero from the no observed effect level. Thus, for our genotoxicity data, a threshold approach is not appropriate.

Summary

In sum, we found that both major dispersants currently used for oil spill cleanup, Corexit 9527 and 9500, are cytotoxic to sperm whale and humpback whale cells. S9-mediated metabolism did not significantly alter the toxicity of dispersants. Corexit 9500 is more cytotoxic than 9527 in both types of whale cells. By contrast, Corexit 9527 was more genotoxic to sperm whale cells than Corexit 9500 and S9-mediated metabolism increase the genotoxicity; however, neither compound was genotoxic to humpback whale cells.

With respect to oil, the water accommodated fraction of oil had low levels of PAHs which did not induce cytotoxicity or genotoxicity in sperm whale or humpback whale cells. When the oil is dispersed using a Corexit compound the levels of PAHs increase significantly. As a result CEWAF is more cytotoxic to both sperm whale and humpback whale cells and S9-mediated metabolism increases the cytotoxic effect. CEWAF alone did not induce a significant amount of genototxicity in either sperm whale or humpback whale cells but S9-mediated metabolism of CEWAF increased its genotoxic effect in both whale cells.

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Wise, C.F., Wise, S.S., Wise, Sr., J.P. Toxicity of chemical dispersants, oil and chemically dispersed oil in sperm whale skin cells (*Physeter macrocephalus*). Proceedings of the Annual Meeting of the International Association of Aquatic Animal Medicine, 44: 143, 2013.

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