Bull. Environ. Contam. Toxicol. (1992) 48:209–215
© 1992 Springer-Verlag New York Inc.
Bulletin of Environmental Contamination and Toxicology

Effect of the Dispersion of Oil in Freshwater Based on Time-Dependent *Daphnia magna* Toxicity Tests

Eric Vindimian, Bernard Vollat, and Jeanne Garric

Laboratory for Ecotoxicology, French Institute of Agricultural and Environmental Engineering Research, CEMAGREF, 3 quai Chauveau, 69336 Lyon Cedex 09, France

The use of dispersants of petroleum is often recommended in accidental aquatic pollution situations where an oil layer is susceptible to reach the banks of a river or a water pond. The petroleum is then emulsified in the water which allows it to be bioavailable for degrading organisms. This bioavailability may be also responsible of an increase of the oil toxicity for the living organisms in the water. Additionally the dispersant itself is potentialy toxic and its release in the environment must be controlled.

The use of petroleum dispersants at sea is well known and has been extensively studied (Nelson-Smith 1968 ; Blackman 1974 ; Nagell et al. 1974). Some procedures for the standardization of the dispersants were developed including toxicity tests on marine organisms (Shelton 1969 ; Laroche et al. 1970 ; Harris et al. 1977 ; Rewick et al. 1981).

Some studies deal with the use of dispersants in freshwater systems and their effects on freshwater living organisms (Rehwoldt et al. 1974 ; Payne 1982 ; Wong et al. 1983 ; Lockhart et al. 1987). The methods used were reviewed by Vandermeulen in 1987. Recommendations about the use of oil dispersants were formulated by the ASTM in 1984 (Allen 1984). It is generally admitted that their use must be limited to the cases where a mechanical elimination of the oil layer is not possible or may be dangerous for the fauna.

In the case of river streams, where the confining of the oil layer is often impossible, the effect of dispersing the oil creates a strong peak of pollution running along the river flow. The living organisms are submitted to a short and intense pollution leading to acute effects.

The purpose of this work is the study of the time dependence of the acute toxicity of oil and dispersants on a sensitive freshwater organism : *Daphnia magna.*

Two different oils were used : a crude oil from the southwest of France and a gas oil free from volatile substances after being equilibrated with atmosphere. Two commercial dispersants were used : British Petroleum Enersperse 1037 and Dasic Freshwater for this study.

Send reprint requests to Eric Vindimian at the above adress.

MATERIALS AND METHODS

The dispersants used were Enersperse 1037 commercialized by British Petroleum and Freshwater by Dasic International limited, Hampshire, U.K. Oil emulsions were prepared using either a polytron homogenizer or ultrasonication during 1 hr at 4°C. The oil drops were monitored with a microscope, the emulsion was homogenous with an average particle size of 2 m. Further dilution of those stock solutions never changed this particle size.

Individuals from the *Daphnia magna* clone of our laboratory were used. Attention was paid to use only daphnids aged 5 to 18 hr to prevent motting during the first 6 hr of the test.

Tests were conducted in closed tubes in order to eliminate water-gas interface and avoid the formation of air bubbles.

The potential effect of closing the tubes was evaluated by running control tests with chromium, in accordance with the French standard AFNOR T90301 (Association Française de Normalisation). The results of the control tests are summarized on Table 1.

Table 1. Control test using potassium bichromate.

Conditions	IC_{50}*, mg/L	CI_{95}
Open 10 mL tubes, 5 daphnids per tube	1.08	0.73-1.50
Closed 5 mL tubes, 4 daphnids per tube	1.08	0.67-1.75
Closed 27 mL tubes, 5 daphnids per tube	0.96	0.66-1.39

* The 24-hr IC_{50} must be within 0.9 to 1.5 mg/L for the validity of the test.

Tests were carefully carried out to prevent the formation of gas bubbles in the tube. When that occurred the test was discarded and run again. The daphnids seemed to float at the water-gas interface which caused a change in the test result and reproducibility. The number of immobilized daphnids was checked at regular time intervals during the test (15, 30, 60, 90, 120, 180, 240, 360, 1440 min.).

The IC_{50} was defined as the concentration of toxicant for which 50% of the daphnids were immobilized and calculated using the probit method (Finney 1971). When the conditions of application of this method were not valid, for instance when immobilization changes from 0% to 100% from one concentration to the concentration immediately above, the IC_{50} was estimated using the geometric mean of the highest ineffective concentration and the lowest effective concentration. The IC_{50} and the concentrations were expressed in µL/L of the oil solution in water.

The IC_{50} versus time, oil type and dispersant was modeled using multiple linear regression. The first step of the regression analysis was the weighing of observations using a robust iterative regression based on the Andrew's sine procedure with a constant value of 2.1. This led to a 0 weigh for the uncalculable IC_{50} values like > 5000 µL/L. The second step was a stepwise regression to discard the insignificant independent variables according to their Student t values. The classical regression procedure was then run using the weighed observations and the relevant set of independent variables. All the calculations were performed using the Number Cruncher Statistical Software on an IBM PC compatible microcomputer. The variables used in that procedure are summarized in Table 2.

Table 2. Variables used in the regression procedure for modeling IC_{50} versus time, oil type and dispersant.

Variables	Type*	Values
Oil	I	-1= crude oil, 0= no oil, 1= gas oil
O2	I	Square of Oil
LogT	I	Natural logarithm of time in hours
Disp	I	-1= Enersperse, 1= Dasic freshwater
OD	I	Interaction Oil X Disp
TO	I	Interaction Time X Oil
TD	I	Interaction Time X Disp
LogI	D	Natural logarithm of IC_{50}

* I : independent variable, D : dependent variable.

RESULTS AND DISCUSSION

As the toxicity of oils can be strongly dependent on the quality of the emulsion, a first comparison was carried out using two different kinds of emulsification procedures. The results of this comparison are summarized on Table 3 ; no significant difference was found between those two different procedures.

Table 3. Comparison between the two types of emulsification of the oil.

Oil	Time, min	IC_{50} Pol*, µL/L	IC_{50} US*, µL/L
Enersperse 1037	30	256	265
	60	258	265
	90	258	265
	120	256	265
	180	256	260
	240	256	260
	360	250	260
	1440	157	161
Dasic Freshwater	120	>5000	>5000
	180	5000	>5000
	240	917	770
	360	310	331
	1440	256	152

* POL means that the oil was emulsified using an Polytron rotating homogenizer, US means that the oil was emulsified using ultrasonication

The comparison of the toxicity of the two dispersants with and without crude oil and atmospheric gas oil is reported on Table 4.

The Dasic Freshwater dispersant is less toxic than the Enersperse especially for short term exposure. The crude oil is far more toxic than gas oil, the latter revealing essentially the toxicity of the dispersant. The graphs Figures 1 and 2 show the comparative toxicity of those dispersants and mixtures versus time.

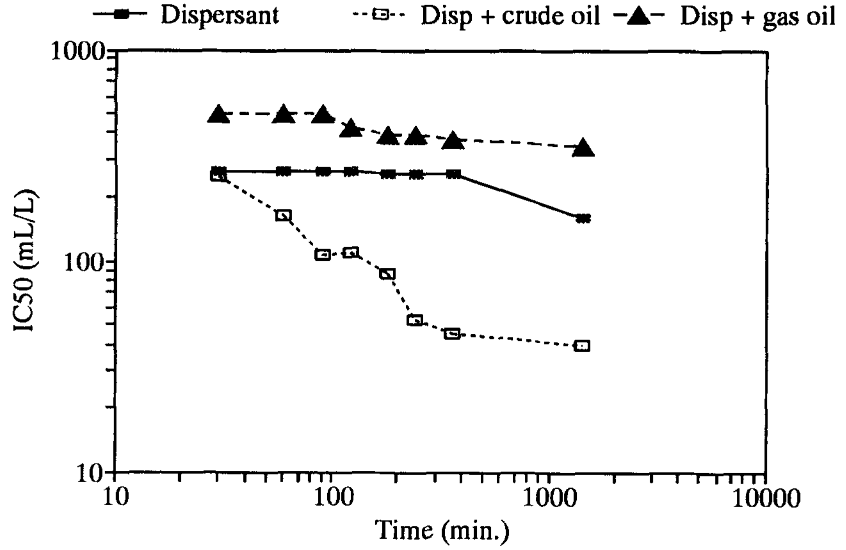

Figure 1. Toxicity of Enersperse.

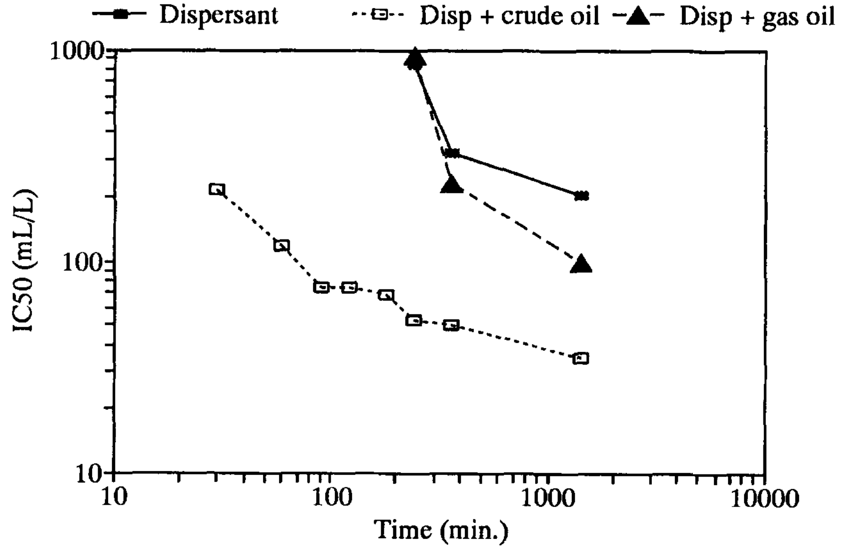

Figure 2. Toxicity of Dasic.

The differential toxicity of the dispersants is strongly time dependent. The Dasic freshwater is only slightly less toxic than the Enersperse 1037 if the usual 24-hr test is used. For a shorter exposure duration the IC_{50} of Dasic is very high whereas the Enersperse is much more toxic. This phenomenon does not allow the choice of the less toxic dispersant on a classical 24-hr toxicity test basis. This emphasizes the interest of the use of different time intervals for the assessment of the toxicity of oils.

Table 4. Toxicity of crude oil and atmospheric gas oil compared with the dispersant toxicity. The crude oil or gas oil was mixed with the dispersant in the proportion 9 to 1. (* means that the IC_{50} was not calculated using the probit method)

Oil	Time min	Dispersant IC_{50}, μL/L	Crude Oil IC_{50}, μL/L	Gas Oil IC_{50}, μL/L
Enersperse 1037	30	261	>249	>500
	60	262	163	*>500
	90	262	107	>500
	120	261	110	424*
	180	258	87	397*
	240	258	53	397*
	360	255	45	374*
	1440	159	40	345
Dasic Freshwater	30	>5000	218	6488
	60	>5000	117	2130
	90	>5000	74	1906
	120	>5000	74	1481
	180	>5000	69	1211
	240	844	53	932
	360	321	50	231
	1440	204	35	99

As the dispersion of oil leads to short term peaks of pollution, the Dasic should be recommended on a toxicological basis. However, this recommendation must take into account the results of effectiveness and degradation tests for the knowledge of the global behavior of the oil for the optimum use of dispersants in case of an accidental oil spill.

The Table 5 summarizes the result of the multiple regression analysis. A strong oil dependence was found, the total regression equation predicted the Log of IC_{50} with an overall correlation coefficient of 0.991.

The regression equation is the following :

$$\text{Log}(IC_{50}) = 6.11 + 1.16\ \text{Oil} - 0.4\ \text{Oil}^2 + 0.2\ \text{Disp} + 0.3(\text{Oil}\times\text{Disp}) - 0.29\ \text{Log}(T)$$

The oil toxicity is the most important variable that must be taken into account in such cases of pollution. This is clearly shown by the coefficient of the variable Oil in the regression equation. The fact that the gas oil is far less toxic than crude oil could be due to the evaporation of toxic volatile fractions of the oil. This fact is of peculiar practical importance since dispersion can occur in field situation after a resting period, the oil layer staying at the surface of water to allow the volatilization of toxic substances. The monitoring of the toxicity of the oil prior to its dispersion could be a helpful in the decision making for an oil spill in freshwater. A simple and easy to handle daphnid short term toxicity test could be this monitoring tool.

Table 5. Result of the multiple linear regression : The student t values where used to calculate the probability that the associated coefficient is equal to zero. The CI95 are the 95% confidence intervals on coefficients.

Variables	Coeff.	CI95%	T value	probability
Intercept	6.11		88	<0.0001
Oil	1.16	1.09 : 1.22	39	<0.0001
Disp	0.20	0.15 : 0.25	8	<0.0001
Log (Time)	-0.28	-0.35 :-0.23	-10	<0.0001
Oil X Disp	0.30	0.24 : 0.36	10	<0.0001
Oil X Oil	-0.40	-0.51 :-0.29	-8	<0.0001

In spite of the described difficulties of manipulation, lethal tests with *Daphnia magna* can give valuable information about short term toxicity of the dispersants and the mixtures oil-dispersant.

Acknowledgment. This study was carried out under the auspices of the CEDRE (Centre de Documentation de Recherche et d'Expérimentations sur les pollutions accidentelles des eaux), the authors wish to thank Mr François Merlin for his collaboration.

REFERENCES

Allen TD (1984) Oil spill chemical dispersants. Research, experience and recommendations. ASTM STP 840 Amer Soc Testing Materials, Philadelphia PA, 465 pp

Blackman RAA (1974) Toxicity of oil sinking agents. Mar Pollut Bull 5:116-118

Finney D (1971) Probit analysis. Cambridge University Press, New York

Harris GW, Doe KG (1977) Methods used by Environment Canada in the testing of oil spill dispersants. Techn Devel Rep EPS-4-EC-77-6. Environment Canada, Ottawa

Laroche G, Eisler R, Tarzwell CM (1970) Bioassay procedure for oil dispersant toxicity evaluation. J Wat Poll Cont Fed 42:1982-1989

Lockhart WL, Danell RW, Murray DAJ (1987) Acute toxicity bioassays with petroleum products : influence of exposure conditions. In: Vandermeulen JH (ed) Oil in freshwater : Chemistry, biology and technology. Pergamon Press, New York, pp 335-344

Nagell B, Notini M, Grahn O (1974) Toxicity of four oil dispersants to some animals from the baltic sea. Mar Biol 28:237-243

Nelson-Smith A (1968) The effects of oil pollution and emulsifier cleansing on marine life in south-west Britain. J Appl Ecol 5:97-107

Payne JF (1982) Metabolism of complex mixtures of oil spill surfactant compounds by a representative teleost (*Salmo gairdneri*), crustacean (*Cabcer irroratus*) and mollusc (*Chlamys islandicus*). Bull Environ Contam Toxicol 28:277-280

Rehwoldt R, Lasko L, Shaw C, Wirrhowski E (1974) Toxicity study of two oil spill reagents toward Hudson river fish species. Bull Environ Contam Toxicol 11:159-162

Rewick RT, Gates J, Smith JH, Mc Carthy JLT (1981) An evaluation of oil spill testing requirements. Proc Oil Spill Conf, Am Petrol Inst, Washington DC pp 5-10

Shelton RGJ (1969) Dispersant toxicity test procedures. Proc Oil Spill Conf, Am Petrol Inst, Washington DC pp 187-191.

Vandermeulen J. H. 1987. Toxicity and sublethal effects of petroleum hydrocarbons in freshwater biota. In: Vandermeulen JH (ed) Oil in freshwater : Chemistry, biology and technology. Pergamon Press, New York, pp 267-303

Wong CK, Strickler JR, Engelhard TFR (1983) Feeding behaviour of *Daphnia pulex* in crude oil dispersions. Bull Env Contam Toxicol 31:152-157

Received February 18, 1991; accepted September 26, 1991.