Bull. Environ. Contam. Toxicol. (1984) 32: 368–376

Environmental Contamination and Toxicology

Effects of Crude Dubai Oil on *Salmo gairdneri* Rich. and *Carassius auratus* L.

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In 1980, owing to the bursting of an oil pipeline, about 663 tons of crude Dubai oil poured into the river Po, the largest Italian river (northern Italy).

The aim of our work was to try and identify, by way of laboratory tests, the lethal concentrations and subsequently calculate the concentration threshold value, below which mortality does not occur (safety dose).

In planning and carrying out the experiments the methodological problems encountered were many, arising from the nature of the toxicants and the lack of bibliographical references about pollution by hydrocarbons of a river ecosystem.

The focal point of the problem is precisely the absence of a working method for tests with hydrocarbons. This makes the preparation of test solutions and the exposure of the animals to the toxic important for the validity of the entire work.

Some authors (Mironov 1967), in fact, propose simply to pour the oil onto the surface of the water, whereas others (Rice et al. 1967) advise an energetic mixing of the oil-water solution for prolonged periods.

MATERIALS AND METHODS

It was decided to carry out the tests with both crude "as it is", and with its water-soluble fraction (SFW), obtained by mixing for 24 hours, 20 liters of normal tap water and 500 grams of crude Dubai at a velocity

of 230 revolutions per minute at room temperature. At the end of mixing it is left to settle for 2 hours, so that the solution stabilizes into two distinct strata: a more or less large deposit on the surface, and below it the SFW, which is taken from the bottom.

As against normal toxicological tests in which the test concentrations are determinated at the beginning, dilutions of the stock solution were used, the actual concentration being determined later, after analysis by infra-red spectrophotometry.

The solubility of the crude in water is connected to the solubility of the compounds, the force of mixing, its duration, the viscosity of the oil and the temperature (McAuliffe 1966, 1969; Anderson et al. 1974; Percy and Mullin 1975; Rice et al. 1976).

In this series of tests it was impossible to obtain concentrations of SFW higher than 54 mg/l at room temperature. The mixing stopped due to "compaction" and this prevented calculation of the LC_{50}s at 24 hours.

Both the series of tests were carried out in "static" conditions, that is without replacing the solution containing the Dubai crude. Plastic test containers were used (capacity 15 liters) immersed throughout the whole test period and kept at a costant temperature (16°C ± 1°C). Carassius auratus L. and Salmo gairdneri Rich., main examples of two families found in the river Po, were used.

Where possible, the data collected were then elaborated statistically by means of the probits method which, by transforming the toxicity curve (% mortality/concentration) into regression lines (mortality in probits/concentration) allows the "average lethal concentration" or LC_{50} to be calculated.

RESULTS AND DISCUSSION

Carassius auratus L., treated with Dubai crude "as it is", resulted in a 48 hr. LC_{50} of 19.89 ml/l. It was not considered appropriate to use higher concentrations in order to obtain noticeable effects at 24h, since a

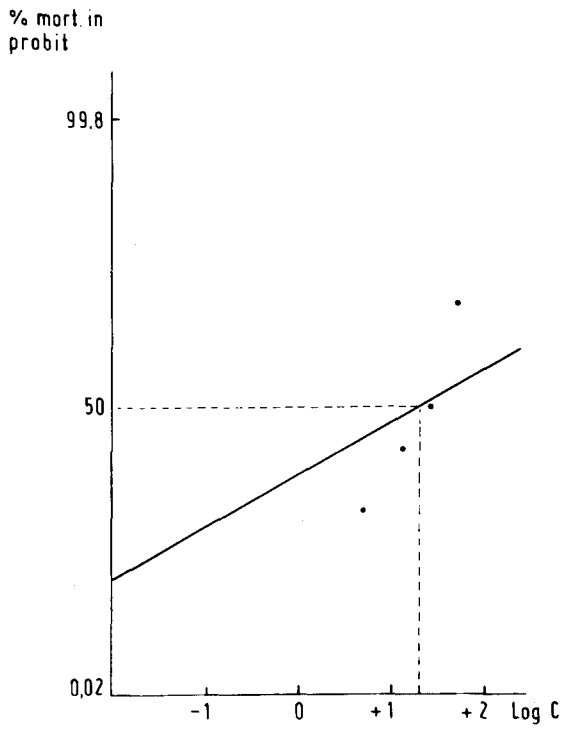

Figure 1. Carassius auratus treated with crude Dubai oil "as it is". Situation at 48h. C= ml/l.

concentration of 100 ml/l is in itself extremely improbable in nature. It is worth noting some behavioural observations made during the course of the experiments in the tanks with concentrations higher than 12.5 ml/l. As soon as the crude was poured onto the surface the fish showed clear symptoms of restlessness such as erection of fins and exaggerated responses to small stimuli (overexcitability), accompained by a clear increase in rate of respiration.

At about 24h, there was a different distribution of the animals inside the tank: they were all on the surface just underneath the veil of crude, whereas in the control the fish were all on the bottom. At 36h typical disorders of balance were present, with rolling into their sides and turning upside down, connected to alterations in the functioning of the swimming bladder and paralysis of the fins. At 48h the situation had deteriorated further, since the fish were immobile on the

surface, turned onto one side and showing scarcely any movement of both fins and mouth. At 72h 100% mortality was present in the tanks. In the tanks treated with lower concentrations the initial state of restlessness and positioning on the surface also occured but after 48h the disorders concerning balance were minimal.

As against the tests with crude "as it is", in all the experiments with the SFW of crude Dubai an initial phase of acute toxicity was present, during which the treated animals showed clear signs of intolerance to the toxicant, such as loss of balance and an increased rate of movement of the operculi and fins. It was in this period that mortality of the treated animals was observed. Having overcome this acute initial phase, the survivors seem to recover completely and behave like the control animals.

This has led to the supposition of a fairly toxic volatile component which causes the acute initial effects and then evaporates.

Therefore a test was set up which took samples of the solution and analyzed them at two-hourly intervals, so as to obtain the trend through time of the concentration of the soluble fraction present in the water. It can be noted from the results given in Table 1 how quickly the SFW disappears.

Table 1. Trend through time of the concentration of the soluble fraction present in the water.

Time in hours	Concentration in mg/l	relative % with t=0 taken as 100%
0	54.1°	100%
1	56.4	104.3%
2	33.4	61.7%
4	28.7	53.1%
6	23.0	42.5%
8	20.1	37.2%
10	18.7	34.6%
12	14.6	27%

° The apparently "wrong" value of the concentration at time 0 may be due to a "dirty" sample, that is in which drops of crude were present.

The disappearance kinetics of the volatile fraction, on the basis of the data available, follow a complex two exponential function model: the first part, for the period between 0 and 2 hours, can be approximated to a linear model (I^{rst} order kinetics), while the second part, from 2 to 12 hours, shows a significantly linear model. The semi-disappearance and total disappearance time of the fraction were also calculated.

The disappearance kinetics (Table 2) can, therefore, be expressed by the relation:

$$C_t = 95.23\ e^{-0.5239\ t} + 38.86\ e^{-0.0794\ t}$$

obtained from the general relation

$$C_t = C_{0,1}\ e^{-K_1 t} + C_{0,2}\ e^{-K_2 t}$$

where

t = time
C_t = concentration at time t
$C_{0,1}$ = initial concentration of phase 1
$C_{0,2}$ = initial concentration of phase 2
e = natural base logorithm
K_1 and K_2 = constants of the two phases

Table 2. Constants of I^{rst} order kinetics model for phase 1 (more volatile fraction) and for phase 2 (less volatile fraction).

Parameters	Phase 1 (0 - 2h)	Phase 2 (2 - 12h)
ln C_0	4.55	3.66 ± 0.041
C_0	95.23 mg/l	38.86 mg/l
K^0	0.5293	0.0794 ± 0.00525
$T_{1/2}$	1^h 20'	8^h 44'
$T_{99/9}$	8^h 47'	58^h

The constants ln C_0 and K phase 1 have no indication of dispersion index (± Standard error) in as far as the calculation was carried out with just two analytical data.

From the gas chromatographical analyses carried out later, the more volatile components turned out to be benzol, toluol and o-m-p-xilol, found in large percentages in the top fraction of the SFW of two samples A and B (Table 3).

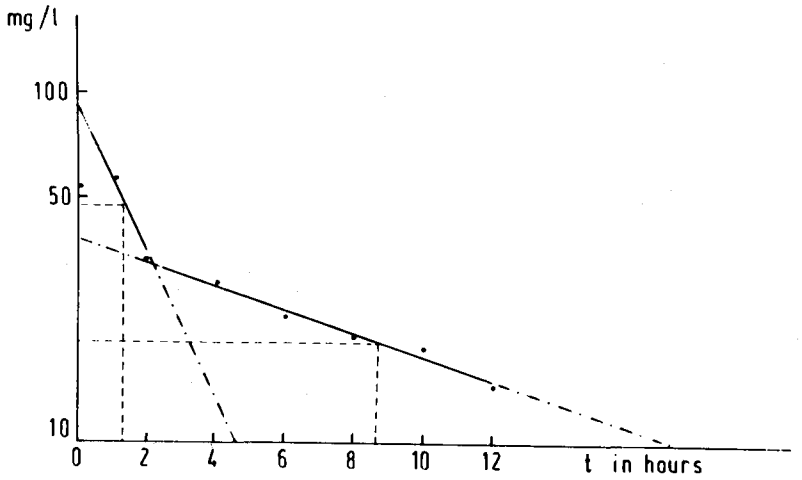

Figure 2. SFW's disappearance kinetics.

Table 3. Components present in the "top fraction" of the aqueous fractions.

Components top fraction	Relative %	A	B
Benzol		54.9	55.1
Toluol		31.3	31.1
Xilol		13.8	13.8

This particular behaviour of the SFW did not allow us to calculate exactly the LC_{50}s under own conditions.

Carassius auratus L. should show such values in the interval between 34.6 and 43.3 mg/l.

A series of experiments were carried out on the first stages of growth of Salmo gairdneri Rich., that is, on fries hatched from the egg a few days previously and therefore with the yolk-sac, on fries which had completed resorption, and on small trout 5 cm in length.

The results of two tests carried out in different conditions are particulary interesting.

In the first the tanks containing the fries with the yolk-sac were oxygenated by means of a porous stone; this device allows the animals to survive, but at the same time makes it easier for the components of the crude in the solution to evaporate. During such an experiment, though initial signs of intolerance to the

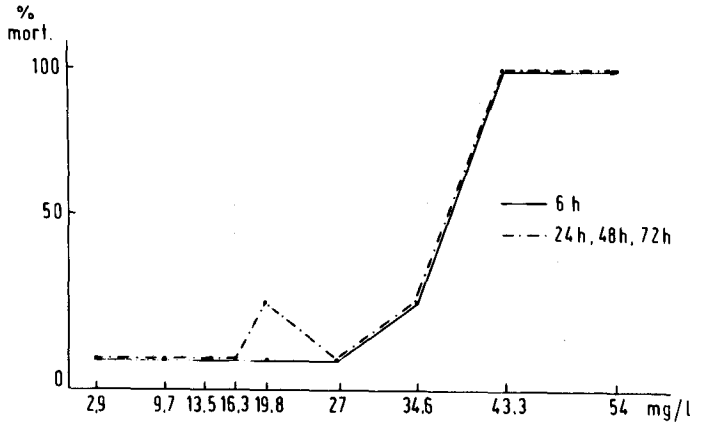

Figure 3. Carassius auratus treated with SFW.

toxic were present, mortality was not observed in the first 24h; later on, on the other hand, the fries died off progressively (70% mortality after 16 days at concentration 29.8 mg/l). At the end of the test the fries in this tank showed a reduced resorption of the yolk-sac with regard to the others, as well as a darker pigmentation, a clear sign that the initial phase of acute toxicity had caused harm to the fish.

The experiment with the 5 cm trout was carried out without forced oxygenation of the tank. After hardly an hour from the start of the test there was already 70% mortality in the tank with a concentration of 19.5 mg/l.

This result indicates that Salmo gairdneri Rich., is undoubtedly very sensitive to this form of pollution.

On the basis of the results obtained it has not been possible to calculate all the LC_{50}s and consequently the safety doses.

Nevertheless, from these first data, the different behaviour of crude Dubai "as it is" and its SFW can be shown. The former, in fact, has a toxic action which is not immediate, with mortality after 24h; there is also an appreciable "covering effect" with regard to the gas exchanges (O_2) caused by the layer of crude on the surface.

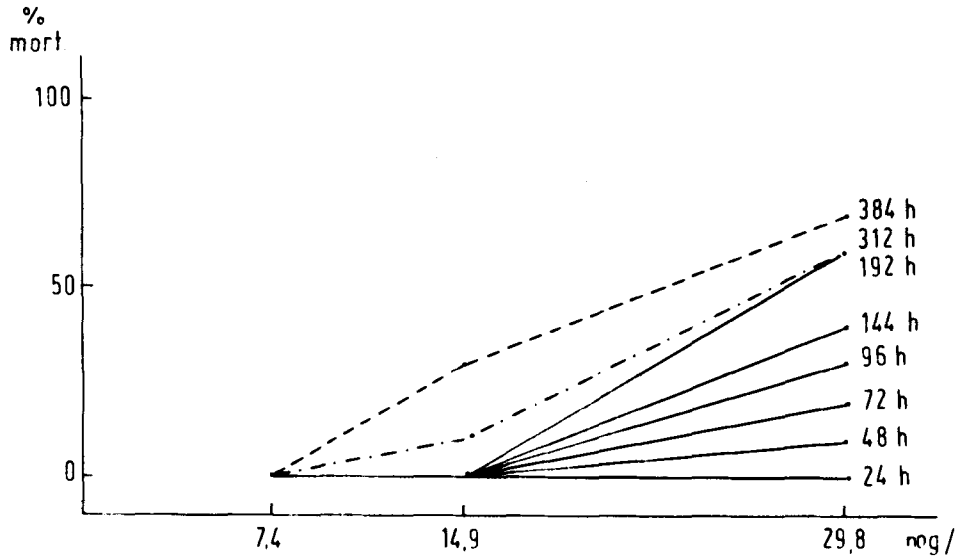

Figure 4. Salmo gairdneri: first experiment with SFW.

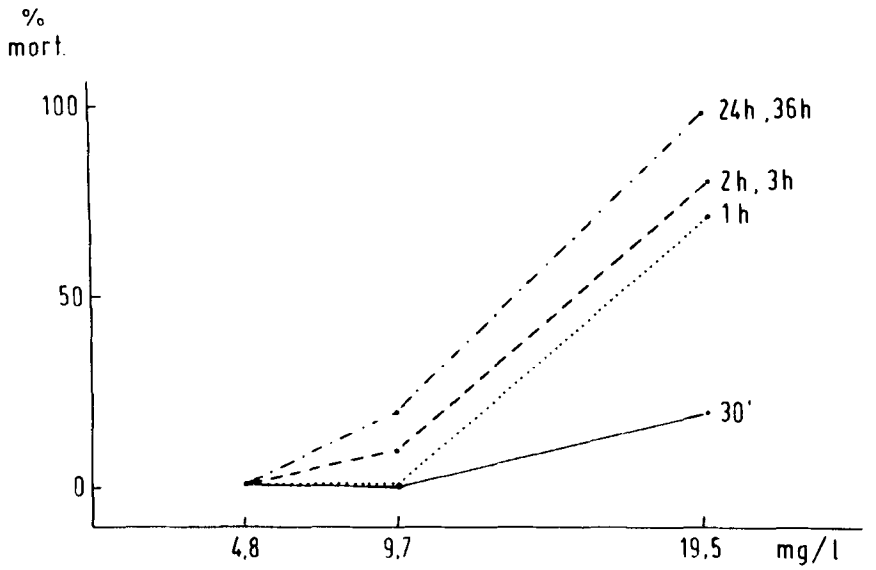

Figure 5. Salmo gairdneri: second experiment with SFW.

The SFW, on the other hand, reaches the height of its perceptible toxic action in the first hours, with an apparent return to normal subsequently.

It would be interesting in some future work to evaluate the toxicity of Dubai crude over longer time periods and show any eventual and permanent harm to the treated animals.

ACKNOWLEDGMENTS

We are grateful to Dr. G. Pacchetti for his statistical analysis and data interpretation.

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Received August 8, 1983; accepted September 15, 1983