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Ecotoxicological Effects of a Nonionic Detergent (Triton DF-16) Assayed by ModFETAX

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We have considered the problem of the effluents of an industry in the field of electronic components whose production processes require the cleaning of the electronic circuit boards. Recently, the traditional use of the chlorofluorocarbons (CFC) for cleaning has been banned and substituted by washing with water and detergents. This has solved the problem of CFC release in the atmosphere, but it has introduced that of detergent release in the water effluents. Detergents appear to be a major ecotoxicological problem; it is well known, in fact, that detergents are extensively used in several other industrial applications as well as personal care and household cleaning products.

In particular, the main constituent of the effluents we were examining was the nonionic detergent Triton DF-16. In view of the extensive use of this product we have tested its ecotoxic effects by modFETAX (modified Frog Embryo Teratogenesis Assay-*Xenopus)* (Bernardini *et al.* 1994) which is a powerful and flexible bioassay for evaluating water soluble toxicants. FETAX (Dumont *et al.* 1993; Bantle *et al.* 1989) has been used for testing single compounds, their synergistic action (Bantle *et al.* 1989; 1990), to estimate water quality (Bantle *et al.* 1989; Dawson *et al.* 1985; 1991) and to evaluate the ecotoxicological efficiency of a water processing plant (Vismara *et al.* 1993).

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MATERIAL AND METHODS

Xenopus, purchased from a local dealer (Rettili, Varese), are maintained in aquaria (Tecniplast, Varese) and fed with beef liver and heart.

In vitro fertilization and embryo selections were performed as described (Bernardini *et al.*. 1994). Briefly, females were injected with human chorionic gonadotropin (Sigma Chemical Co., St. Louis, Missouri). About 16-hr after, females were made to lay eggs in plastic Petri dishes. Eggs were immediately fertilized with sperm suspension obtained by mincing the testes in cold FETAX solution, whose composition (in mg/L) was: NaCl 625, $NaHCO_3$ 96, KCl 30, $CaCl_2$ 15, $CaSO_4 \cdot 2H_2O$ 60 and $MgSO_4$ 70 (Dawson *et al.* 1987).

Successful fertilization is easily detected when after a few minutes all the eggs are oriented with the dark side (animal pole) up. A first screening, performed 2-hr post fertilization (p.f.), allows to discard the 'bad' eggs and the unfertilized ones. This screening is followed by a second one (6-hr p.f.) where all the embryos with some abnormalities are eliminated and eventually replaced to leave each Petri dish with 8 normal embryos. Assays were run in duplicate or in triplicate.

The tested concentrations of Triton DF-16 ranged from 10 to 50 µL/L and were obtained by dilution from a stock solution prepared fresh for each experiment. Negative controls, in FETAX solution alone, were run concurrently. All test solutions were administered 8-hr p.f. Each day the solutions were renewed and the dead embryos were removed. Embryos were kept in a thermostatic chamber at 23°C $\pm$ 0.5. At 120-hr embryos were evaluated for mortality (the absence of heartbeat and motility was used as indicator of death); the surviving embryos were anaesthetized with MS 222 (Sigma Chemical Co., St. Louis, Missouri) and evaluated for gross malformations; immediately afterwards, embryos were fixed (2% paraformaldehyde in 0.1 M cacodylate buffer at pH 7.3). For each concentration, the number of dead and surviving malformed embryos were recorded, as were the number and types of specific malformations.

The recorded malformation types were axial abnormalities, edema (generalized, cardiac, abdominal and optic), loose or

improper gut coiling, ocular abnormalities (reduction in eye size, lens extrusion, oval shaped eyes) and cardiac malformations (abnormal expansion of the ventricle, failure of the heart tube to coil properly). Grossly malformed embryos with multiple malformations were classed as "monsters". Only the embryos scored as normal were then measured.

Head-tail lengths were measured from the images acquired by a system composed of a charge coupled device (CCD) camera attached to a Stemi SV 6 Zeiss stereomicroscope and a personal computer equipped for image analysis (Wanalyst software, Eidosoft, Milano).

The relationship between Triton DF-16 concentration and percentage of dead or malformed embryos was investigated by probit analysis (Finney 1971). The effective concentrations at 120-hr p.f. were designated, depending on the case, lethal (LC) or teratogenic (TC) concentration (Bernardini *et al.* 1994). The Teratogenic Index (TI) value, useful in estimating the teratogenic risk associated with the compound (Dawson *et al.* 1987), was defined: $TI_{50} = LC_{50} / TC_{50}$. Growth retardation analysis has been carried out using an ANOVA model with mixed effects (Bernardini *et al.* 1994). The statistical analysis was performed with the SAS software package.

RESULTS AND DISCUSSION

FETAX is a powerful test for the presence of developmental toxicants (Dumont *et al.* 1983; Bantle *et al.* 1989; Plowman *et al.* 1991; Sunderman *et al.* 1991). We have used an improved version (Bernardini *et al.* 1994) of this test (modFETAX) to evaluate toxic effects of Triton DF-16, a nonionic surfactant.

Experiments with mortality and malformation rates in the controls greater than 20% were discarded. The overall mortality and malformation rates of the controls of the analyzed assays were 5% (over 119 embryos) and 6% (over 84 embryos), respectively.

The expected frequencies of dead and malformed embryos estimated by probit analysis for Triton DF-16 concentration (and their 95% fiducial limits) are shown in Fig. 1. From a LC_{50} of

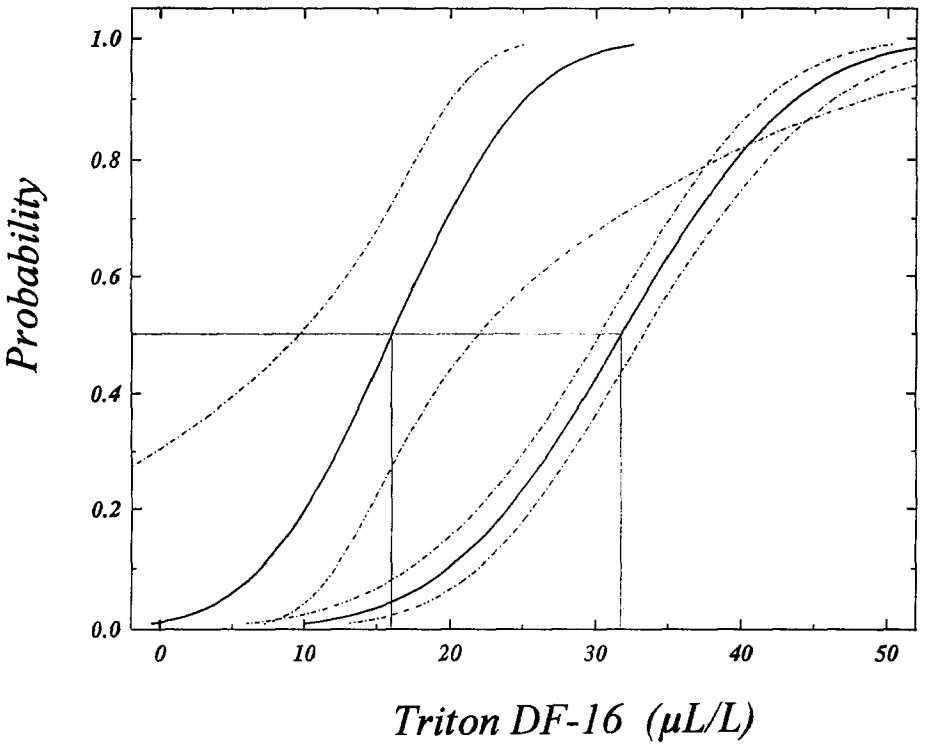

Figure 1. Concentration-response curve for *Xenopus* embryos exposed to Triton DF-16 elaborated by probit analysis; malformations (left curve), mortality (right curve), fiducial limits (dotted lines).

31.74 µL/L and a TC_{50} of 16.01 µL/L, a TI_{50} of 1.98 is derived. This indicates that the chemical has to be considered weakly teratogenic (Dawson *et al.* 1987).

The mean length of control embryos was 9.85 mm (SE ± 0.38, 65 embryos), the mean length of embryos exposed to 10 µL/L of Triton DF-16 was 9.53 (SE ± 0.31, 56 embryos). The difference between mean length of control embryos and those exposed to Triton is significant ($P<0.05$) and the first order interaction between concentrations and females was significant ($P<0.05$). The 10 µL/L concentration of Triton DF-16 significantly reduces the embryo length.

In the life cycle of any organism, development is certainly a weak link and this has often been disregarded in designing tests

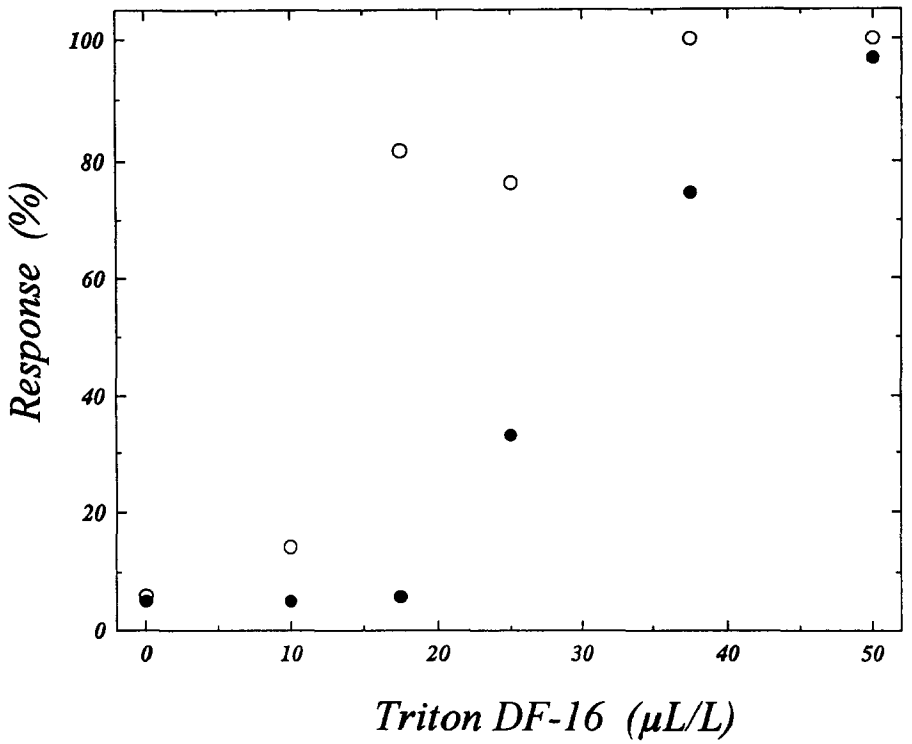

Figure 2. Experimental data of mortality (filled dots) and malformations (empty dots) for *Xenopus* embryos exposed to Triton DF-16.

for the assessment of the ecotoxicological risks. ModFETAX, on the contrary, proved to be a powerful test useful for detecting the presence of developmental toxicants in the environment, for assessing the water quality of a hydrographic basin, as well as for evaluating a water purification system.

Our data show that the LC_{50} for Triton DF-16 is 31 μL/L and that the TC_{50} is 16 μL/L. Moreover, a concentration of 10 μL/L caused a significant growth retardation. As lethal and teratogenic concentrations are similar, as indicated by the TI, the chemical can be considered only slightly teratogenic for *Xenopus* embryos. It is, however, clear that waters containing as

low as 10 μL/L of Triton DF-16 can impairing development and perhaps the survival of this species.

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