Bull. Environ. Contam. Toxicol. (1994) 52:292–297

Environmental Contamination and Toxicology

Effect of Components of the Textile Mothproofing Process on Three Freshwater Microalgae Species

M. C. Riva, B. Valles

Textile Research Institute, Polytechnical University of Catalonia, C/ Colom 15, 08222 Terrassa, Spain

Received: 14 October 1992/Accepted: 20 July 1993

Mothproofing process is characterized by the utilization of insectical and surface active agents. Insectproofing agents are often applied mainly to wool, as this can be attacked by moths and carpet beetles. The process can be carried out during dyeing. Highly effective products based on dieldrin were popular in the 1960s but these are now environmentally unacceptable and, in Australia, dieldrin-resistant moth strains have developed.

Finishes based on the application of a pyrethroid-type agent have been recently introduced. Pyrethroids are products of synthesis with a similar insecticide activity as natural pyrethrines. They are halogenated esters (Cl/Br/F) composed of a cyclopropanocarboxylic acid radical with 1 or 2 assymetric carbons and 1 alkymetric alcohol radical of cyclopenthenolone or phenoxybencyl, with or without assymetric carbon (Piedrola 1982). Pyrethroids have many desirable properties such as high toxicity to insects, relatively low toxicity to mammals, photostability and high biodegradability. Steven et al. (1989) reviewed existing data about pyrethroid toxicity in mammals, birds, fish, amphibians, and invertebrates.

Since permethrin was identified as a potential insecticide (Elliott et al. 1978), studies have evaluated its effects on invertebrates in the laboratory, in situ bioassay and field experiments. Results of these studies show invertebrates to be highly sensitive to permethrin and also that this insecticide may cause severe perturbations of benthos.

Surfactants are large volume chemicals used in detergents, household cleaning and personal care products and, to a lesser extent, in pesticides, herbicides, paints, plastics, and in the mining, oil, and textile industries. The toxic effects of representative surfactants on aquatic life have been determined and summarized in greater detail for animal test species rather than for aquatic vegetation (Lewis 1990), but no studies have been found for some commercial textile products.

The objective of the present study was to determine the effect of some auxiliaries of the textile mothproofing process to three freshwater microalgae species, since no toxicity data exist for these compounds.

MATERIALS AND METHODS

The studied compounds were a textile finishing of permethrin and a hexahydropyrimidine derivative with anionic characteristics (Mitin AL) (Wimbush 1985) and two tensoactive products Avolan UL75 (alkylarylaminpolyglic olic eter) and Albegal B (amino-hydroxyethyl

Correspondence to: M. C. Riva

fatty acid derivative) that act as dyeing auxiliaries with amphoteric character. Chlorella vulgaris CCAP 211/11B, Scenedesmus subspicatus CCAP 276/20 and Selenastrum capricornutum CCAP 278/4, from Culture Collection Algae and Protozoa (Cumbria, U.K.) were used.

The determination of the alga growth inhibition was carried out by the OEDC method 201 (1984) with some modifications. The algal growth was carried out under two light conditions, in a bath culture system, with a 100 mL solution volume, in test flasks of 250 mL. They were shaken and placed in a controlled temperature chamber (T = 22 ± 2 ºC), in the first case under continuous lighting of 2500 lux, and a 8hrs light - 16 hrs darkness photoperiod in the second trial, and all operations were carried out under sterile conditions in order to avoid contamination with bacteria or other algae. The medium employed was maintained for total hardness (0.5 mM), dissolved oxygen (7.8-8.1 mg/L) and pH (7.8±0.2).

Exponentially-growing cultures (10^3 cells/mL) of Chlorella vulgaris, Scenedesmus subspicatus and Selenastrum capricornutum were exposed to various concentrations of the test substance. The inhibition of growth in relation to a control culture was determined over a fixed period of 72 hr. To determine cell concentrations a Shimadzu UV-240 (PR-1) Spectrophotometer with 1cm light path cuvettes and a Neubauer counting chamber were used, and the relation between the optical density and the cell number was established for each species. In the treatments for the concentration range, at least five concentrations of each product arranged in a geometric series were selected, and four replicates at each test concentration and twice that number of controls. Filtered algal medium was used to determine the background as a blank when using spectrophotometer. The pH was measured at the beginning of the test and at 72 hr. The growth rate was obtained by linear regression. Percentages of growth inhibition were calculated and inhibitory concentrations 72 hr EC 50 were established according to Finney (1964). Results were compared by ANOVA using the Statgraphics software.

The quantification of algal pigments was carried out by determination of chlorophylls and total carotenoids by spectrophotometric techniques (Trichromatic Method). The pigments were extracted from the three alga species (Chlorella vulgaris, Scenedesmus subspicatus and Selenastrum capricornutum) during the growth inhibition test. After filtration of algae and media by 0,45 µm polycarbonate membrane filter (Nucleopore 25 mm), 1 mL magnesium carbonate (1%) suspension was added before centrifuging. The concentrated samples were stored frozen in a desiccator in the dark when extraction was delayed. In a 15 mL centrifuge tube, 8 mL of acetone (90%) was added to the sample and macerate and filter were dissolved. The extraction of the pigments was carried out in the cooling unit during 20 hr in the darkness, shaking the tubes in the first and the second hours. When tubes were conditioned in a room temperature they were carried with acetone (90%) to a 10 mL of a final volume and centrifugated 5-10 min to 3000-4000 rpm. For the quantification of the pigments a Shimadzu UV-240 (PR-1) Spectrophotometer was used with 1cm light path cuvettes at the maximun absorption wavelength: 663, 645 and 630 nm for chlorophyll a, b, and c, and 480 and 510 nm for carotenoids.The optical density reading at 750 nm serves as a correction for turbidity. The quantity of pigment was calculated according to Amer. Publ. Health Assoc. (1985), being the quantity of pigment per volume unit of sample as follows:

$$Q\ (\text{mg pigment}/m^3) = CxV_1/V_2$$

where C is the pigment concentration in the extract (mg/L), V_1 and V_2 are the volume of the extract (L) and the sample (m^3) respectively. Pigment concentrations were obtained previously by the following equations:

$$Ca = 11.64\ D663 - 2.16\ D645 + 0.10\ D630$$
$$Cb = 20.97\ D645 - 3.94\ D663 - 3.66\ D630$$
$$Cc = 54.22\ D630 - 14.8\ D645 - 5.53\ D663$$

where Ca, Cb, Cc are the concentrations of chlorophyl a, b and c, respectively, in the extract, and D663, D645 and D630 are optical densities at the respective wavelengths.

RESULTS AND DISCUSSION

The physiological condition of the control cultures was controlled by the relation between cell number and optical density at the maximum wavelength of each culture. Algae biomass declined with Mitin AL, Avolan UL75 and Albegal B concentrations in our toxicity tests (Table 1).

Table 1. Toxicity values from green algae Chlorella vulgaris, Scenedesmus subspicatus and Selenastrum capricornutum exposed to textile components Mitin AL, Avolan UL75 and Albegal B.

Component	72-hr EC50 (mg/L)					
	Chl. vulgaris		Sc. subspicatus		Sel.capricornutum	
	Cont. Light	8L/16D	Cont. Light	8L/16D	Cont. Light	8L/16D
Mitin AL	2.0 ±0.18	1.12 ±0.22	1.0 ±0.37	0.4 ±0.08	0.9 ±0.18	0.5 ±0.16
Avolan UL75	11.0 ±1.82	1.35 ±0.23	5.12 ±1.16	0.9 ±0.29	7.0 ±0.7	5.97 ±0.25
Albegal B	0.009 ±0.002	0.019 ±0.008	8 ±0.25	5.85 ±0.71	9.25 ±1.7	3.95 ±0.31

Values are expresed as mean of 4 replicates ± SD.

The result of the ANOVA of table 1 indicated that the three studied factors influenced the response significatively. The interactions product-species and product-lighting conditions had a significant influence in the response, but species did not interact with lighting conditions (Sig. level = 0.2373 >>0.05). No differences could be deduced between Avolan UL75 and Albegal B toxicity, but the response EC50 (mg/L) induced by them is clearly greater than Mitin AL as a consequence of this, Mitin AL was more toxic than Avolan UL75 and Albegal B for Chlorella vulgaris, Scenedesmus subspicatus, and Selenastrum capricornutum. All species showed different behavior, not being possible to discern between the last one and Selenastrum capricornutum, but the responses of Chlorella vulgaris and Selenastrum capricornutum were clearly different.

The behavior of the species was different for each product, and the effect of the product was a function of the light condition, being the products more active in continuous lighting than in a 8L/16D photoperiod.

In fact when algal species are exposed to several effluents, it is possible that individual chemicals of the wastewater or all its components could affect the algal growth. In our case, after adding varying concentrations of some textile auxiliaries from the textile mothproofing process to three microalgae species, variable toxicity to 72hr has been obtained (Table 1).Alga exhibited a dose-response relationship between growth and increased Mitin AL, Avolan UL75 and Albegal B concentrations. The algal data give an indication of possible chronic effect levels for these compounds. In addition, the inhibition effects could have serious consequences for the food chain if they occurred in

a natural system. There are no other aquatic toxicity data available for Avolan UL75 and Albegal B. It is known that different kinds of Mitin with a LC50=1 mg/L to the zebrafish Brachydanio rerio (Ciba-Geigy, 1986) which is similar in our experiment with alga.

The three chlorophylls a, b and c,and the carotenoid pigments (carotens and xanthophylls),in both control cultures and that exposed to the median effective concentration (72-hr EC50) of these textiles components in photoperiod conditions have been determined (Table 2).

Table 2. Algal pigment contents (µ/L) in Chlorella vulgaris, Scenedesmus subspicatus and Selenastrum capricornutum in control and treated cultures (72 hr) with Mitin AL, Avolan UL75 and Albegal B with the respective EC50 in photoperiod conditions presented in Table 1.(Values are expressed as mean of 4 replicates ± SD)

Products	Species	Chlorophyl a µg/l	Chlorophyl b µg/L	Chlorophyl c µg/L	Caretenoids µg/L
Control	Ch. vulg.	238.85±1.28	52.27±1.38	111.9 ±1.99	111.17±1.19
	Sc. subs.	260.95±2.87	69.35±2.41	40.65±2.48	219.1 ±2.29
	Se. capr.	364.77±3.41	64.8 ±2.54	8.57±1.96	317.87±5.17
Mitin AL	Ch. vulg.	200.02±6.62	55.2 ±8.28	41.22±2.37	113.32±3.17
	Sc. subs.	128.52±1.56	5.65±2.01	64.8 ±4.11	181.7 ±2.63
	Se. capr.	361.32±2.54	66.45±3.66	11.6 ±2.02	292.2 ±5.58
Avolan	Ch. vulg.	185.92±5.66	69.77±2.53	43.4 ±3.87	113.7 ±3.36
UL75	Sc. subs.	131.95±2.8	15.3 ±2.74	60.2 ±7.51	150.5 ±5.05
	Se. capr.	362.75±8.98	72.65±2.86	3.8 ±2.15	314.3 ±10.3
Albegal	Ch. vulg.	325.05±4.88	71.1 ±4.61	51.57±5.01	191.5 ±5.46
B	Sc. subs.	140.95±6.43	27.97±5.30	48.27±3.09	125.8 ±6.06
	Se. capr.	416.1 ±9.93	83.42±5.67	10.1 ±2.12	331.0 ±8.92

The ANOVA of Table 2 showed that the three factors, product, species and pigment had significant influence in the response and all interactions were significant too. Mitin AL and Avolan UL75 had similar effect decreasing the level of pigments in relation to the control. Albegal B increased the pigments content being higher than controls. All species had different behavior. Scenedesmus subspicatus response exhibited an important reduction on pigment levels, whereas Chlorella vulgaris and Selenastrum capricornutum presented an increase of them in relation to the controls. Chlorophyll c was the only one that had high levels in all species and for the three treatments. Chlorophyll b and carotenoids with similar values had lower levels that controls, and a reduction of chlorophyll a with values below the controls was found.

Biomass in treated species was affected by the presence of the toxic agent. Results found in this study agree with those of Hoda Abou-Waly (1991) who measured the algal biomass using chlorophyll a content. These authors found that for Selenastrum capricornutum effects were generally related to herbicide dose. Singh and Gaur (1988) showed that low concentrations of crude oil stimulated the algal activity while higher concentrations were inhibitory. The extent of inhibition was dependent on the amount of agent in the medium. Laughlin et al. (1981) found that low doses of jet fuel (mixed hydrocarbons) initially enhanced the growth of algae, followed by a decrease in growth. The amounts of chlorophyll a content in our untreated species ranged from 200 to 300 mg/m^3 (0.2-0.3 µg/mL) which are a little smaller than levels given by Hersh and Crumpton (1989) who found chlorophyll a amounts ranged from 0.9 to 7.9 µg/mL from Chlorella sp. and other species of Chlorococcales.

The compounds considered in this study seemed to be as toxic as some chlorinated compounds which were studied before (Riva & Vallés, 1990) with Chlorella and Scenedesmus. Algal species appear to be the most sensitive to many halogen-containing products. Some esters may cause toxicity by altering the permeability of the membranes (Bentley et al. 1978), thus causing an immediate effect on these single-cell species. Toxicity studies which some auxiliaries of one textile process indicate a more mixed sensitivity trend. There are chemicals that can act changing or modifying biochemical reactions, like the pigment synthesis, and the morphology of the cell structures could be altered too.

Pigment content may be a satisfactory toxicity assessment method, but physiological effects also need to be known. Products are normally used in small quantities in the processing of the wool. The authors think adequate to study the persistence for absorption to sediment.

The range of toxicities and effects reported in this study indicates that Mitin AL, Avolan UL75 and Albegal B could be a problem in most aquatic environments if they were not controlled. Clearly, there is an obligation of continuing the research in this area if the actual risk posed by specific discharges to the aquatic environment is to be understood.

Acknowledgments. This work received financial support from Dirección General de Investigación Científica y Técnica (DGICYT) del Ministerio de Educación y Ciencia PB87-0180. Spain.

REFERENCES

American Public Health Association (1985) Standard methods for examination of water and wastewater analysis.16th.ed. APHA.AWWA and WPCF Washington, DC

Bentley RE, JW Dean, SJ Ellis, GA Leblanc, S Sauter, KS Buxton, BH Light (1978) Laboratory evaluation of the toxicity of nitroglycerin to aquatic organism. Final report, AD A061739. Defense Technical Information Center. Alexandria

Ciba-Geigy (1986) Working Instruction 6374D, Switzerland

Elliott M, Janes NF, Potter C (1978) The future of pyrethroids in insect control. Ann Rev Entomol 23: 443-469

Finney DJ (1964) Probit analysis. A statistical treatment of the sigmoid response curve. Cambridge University Press, London

Hersh CM and WG Crumpton (1989) Atrazine tolerance of algae isolated from two agricultural streams. Environ Toxicol Chem 8: 327-332

Hoda Abou-Waly, MM Abou-Setta, HN Nigg and LL Mallory (1991) Dose-response relationship o f Anabaena flos-aquae and Selenastrum capricornutum to atrazine and hexazinone using chlorophyll (a) content and ^{14}C uptake. Aquat Toxicol 20: 195-204

Laughlin RB , J Ng and HE Guard (1981). Hormesis: a response to low environmental concentrations of petroleum hydrocarbons. Science 211: 705-707

Lewis MA (1990) Chronic toxicities of surfactants and detergent builders to algae: A review and risk assessment. Ecotoxic Environ Saf 20: 123-140

OECD (Organization of Economic Cooperation and Development) 201 method (1984). Alga growth inhibition test. Draft 26.7.83. Paris

Piedrola-Gil G and G Piedrola-Angulo (1982) Importancia de los nuevos piretroides sintéticos. Ann Rev Acad Nac Med 99: 231-273

Riva MC and B Vallés (1990) Efectos de compuestos orgánicos halogenados en Chlorella vulgaris y Scenedesmus subspicatus. Ac. II Congreso Nacional de Acuicultura: 633-637.

Singh AK and JP Gaur (1988) Effect of assam crude on photosynthesis and associated electron transport system in Anabaena doliolum. Bull Environ Contam Toxicol 41: 776-780

Steven P, I Bradbury and JR Coats (1989) Comparative toxicology of the pyrethroid insecticides. Reviews of Environ Contam and Toxicol 108: 133-177

Wimbush JM (1990) The chemical analysis of insect-resist agents on wool. Part 2-pyrethroids. JSDC 106: 241-244.