1 Research supported by the Division of Human Genetics, K.U. Leuven, Belgium. The Zairian ONRD permitted a stay at IRSAC-Mabali. The Department of Human Genetics, University of Amsterdam, Netherlands, provided laboratorium facilities for cytophotometric analysis. I thank J. Bylsma, M. Kibishwa, F. Koperdraad and H. Van den Berghe for advice.

2 D.F.E. Thys van den Audenaerde, Bull. Acad. r. Sci. O.-M. *VII,* 222 (1961).
3 P.H. Greenwood, D.E. Rosen, S.H. Weitzman and G.S. Myers, Bull. Am. Mus. nat. Hist. *131,* 339 (1966).
4 W.A. Gosline, in: Functional morphology and classification of teleostean fishes, p. 111. University Press of Hawaii, Honolulu 1971.
5 D.E. Rosen and P.H. Greenwood, Am. Mus. Novit. *2428,* 1 (1970).
6 T.R. Roberts, in: Interrelationships of fishes, p. 377. Ed. P.H. Greenwood, R.S. Miles and C. Patterson. Academic Press, London 1973.
7 R. Hinegardner and D.E. Rosen, Am. Nat. *106,* 621 (1972).
8 R. Arai, K. Nagaiwa and Y. Sawada, Jap. J. Ichthyol. *22,* 241 (1976).
9 A.D. Kligerman and S.E. Bloom, J. Fish. Res. Bd Can. *34,* 266 (1977).
10 A. Levan, K. Fredga and A.A. Sandberg, Hereditas *52,* 201 (1964).
11 H.G. Schiemer, Z. wiss. Mikrosk. *69,* 244 (1969).
12 A.G.E. Pearse, in: Histochemistry, 2nd ed., p. 201. J. and A. Churchill, London 1960.
13 A. Post, Z. Zool. Syst. EvolForsch. *3,* 47 (1965).
14 R.P. Nayyar, Genetica *37,* 78 (1966).
15 A.B. Chiarelli, in: Cytotaxonomy and vertebrate evolution, p. 205. Ed. A.B. Chiarelli and E. Capanna. Academic Press, London 1973.
16 J.J. Scheel and B. Christensen, Trop. Fish Hobby. *19,* 24 (1970).
17 W.M. Howell, Copeia *1972,* 191.
18 W.G. Ridewood, Zool. J. Linn. Soc., Lond. *29,* 252 (1905).
19 D.F.E. Thys van den Audenaerde, Ann. Mus. Afr. cent. Sér. 8vo (Sci. zool.) *103,* 100 (1961).

Biodegradability of Tioctilate

P. Pipyn, C. Beaudet[1] and W. Verstraete

Laboratory of General and Industrial Microbiology, State University of Gent, Coupure, 533, B-9000 Gent (Belgium), 16 May 1978

Summary. The biodegradability of Tioctilate (octylthiobenzoate), a new pesticide, has been examined by means of 5 tests. The compound appears to be susceptible to microbial metabolism.

Tioctilate (octylthiobenzoate) has recently been established as an efficient chemical to eradicate mites, lice and even *Trichophyton.* The compound appears to have a very low oral toxicity (LD_{50} for rats = 5.9 g/kg) and is little or not resorbed through the skin. The purpose of this work was to examine the microbial degradability of Tioctilate in order to evaluate its environmental acceptability.

Materials and method. Tioctilate is a colourless liquid, slightly soluble in water (2 mg/l at 20 °C), stable towards diluted acids and alkali. Water samples are extracted with heptane, the organic layer is evaporated under reduced pressure to dryness and the residue is redissolved in a known quantity of heptane. The latter solution is subsequently analyzed by gas chromatography under the following conditions: column: ⌀ ⅛", 5 foot length, SE 30 10% on chromosorb W-HP 80–100 mesh, 220 °C; carrier gas: nitrogen 30 ml/min; FID; retention time: 7 min.

The biological oxygen demand (BOD_5^{20}-test) was determined by means of the conventional bottle test[2]. The other 4 tests were performed as described in detail by Voets et al.[3]. The 2 aerobic tests were performed according to the OCDE-methods for the biodegradability testing of detergents In the minimal test (MM-test), the biocide is dissolved in a mineral solution with the following composition (g/l): a) KH_2PO_4, 8.5; K_2HPO_4, 21.75; $Na_2HPO_4 \cdot 2H_2O$, 33.4; NH_4Cl, 1.7; b) $MgSO_4 \cdot 7H_2O$, 22.5; c) $CaCl_2$, 27.5; d) $FeCl_3 \cdot 6H_2O$, 0.25. To prepare 1 l of the MM-medium, add 1 ml of each of the solutions a–d to 1 l of distilled water. 1 l of this solution was incubated in a 2.0 l Erlenmeyer flask on a rotary shaker (120 rpm). The flask was inoculated with 1.0 ml of soil extract and incubated open to the air. To prepare the soil extract, 10 g of a fertile field soil were suspended in 100 ml tap water and gently mixed. The suspension was filtered through a 595-Whatman paper filter, the filtrate being used as inoculum. Evaporation losses were regularly adjusted with distilled water. To detect losses of volatile substances, a control flask containing the sterile-filtered biocide solution was also incubated. The synthetic sewage had the following composition (mg/l): peptone, 160; meat extract, 110; urea, 30; NaCl, 7; $CaCl_2 \cdot 2H_2O$, 4; $MgSO_4 \cdot 7H_2O$, 2; tap water 1 l. The synthetic sewage passed through the aeration vessel at a rate of 1 l/h. To start up the activated sludge, the apparatus was fed during a 2-week period with synthetic sewage devoid of biocides. During the actual trials, the concentration of the test chemical was monitored daily in the influent and effluent. Since the test measures the biodegradation of the biocide in a complex organic medium, it is referred to as Organic Medium-test (OM-test).

The media used in the 2 anaerobic tests were the same. However, in the anaerobic MM-test, a 1-l Erlenmeyer flask was filled up to the rim with the MM-solution. The flask was subsequently incubated at 22 °C in an anaerobic chamber (BBL gaspack jar). In the anaerobic OM-test, 1 l of the OM-medium was inoculated with 1 ml of soil extract and incubated in the anaerobic chamber. The chemical oxygen demand (COD) was measured by the dichromate method according to the Standard Methods[5].

Results. The BOD_5^{20}-test revealed that an aqueous solution of Tioctilate with an initial COD of 4.70 mg/l, had a net

Die-away of Tioctilate in mineral medium under aerobic conditions

Days	Percentage remaining					
	Inoculated flasks			Sterile control		
	A	B	Average	A	B	Average
0	100*	100	100	100	100	100
5	1.7	5.5	3.6	–	–	–
10	2.9	2.2	2.5	–	–	–
15	2.5	0.4	1.4	140	65	102
20	0.1	–	0.1	144	72	108

* The percentages refer to the value detected at the onset of the die-away test.

oxygen consumption of 0.32 mg/l in a period of 5 days. From this, it can be calculated that about 10% of the organic carbon had been converted to CO_2 (tertiary biodegradation). However, chemical analyses of the incubated samples indicated that 51% of the product had disappeared and thus undergone primary biodegradation.

In the aerobic MM-test, the pesticide is dissolved in a mineral medium inoculated with soil and incubated at room temperature on a rotary shaker (cf. the screening test of the OCDE-method for biodegradability testing of detergents). A rapid disappearance of Tioctilate was observed (see table). It should be added that the sterile control A was incubated in the dark, while the sterile control B was incubated in the light. Hence, Tioctilate is not very susceptible to photolysis. In the anaerobic MM-test, the samples are incubated under strictly anaerobic conditions. Under these conditions, only about 40% of the product disappeared in a period of 20 days.

The aerobic OM-test corresponds to the standard activated sludge test for detergents[4,6]. A rapid and complete (99.8%) biodegradation of Tioctilate was observed, even without a preliminary adaptation of the sludge system. No apparent disturbance of the microbial community could be detected upon introduction of the product to the activated sludge bassins. For the anaerobic OM-test, a disappearance of up to 80% was noted after 5 days, while 90% of the product was biodegraded after 20 days.

Discussion and conclusions. The various experiments indicate that Tioctilate is quite susceptible to microbial metabolism provided aerobic conditions prevail. Hence, this product should rapidly disappear from oxygen-rich environments such as aerobic waste treatment systems and surface waters and soils. However, it must be pointed out that the results of the die-away tests reflect the primary biodegradation of the compound. The BOD-test, which indicates the complete conversion of the compound to carbon dioxide and water, suggests that the tertiary biodegradation of Tioctilate proceeds rather slowly. Nevertheless, in view of the nonxenobiotic character of the immediate metabolites of Tioctilate (i.e. benzoic acid and octanethiol), one can expect a normal albeit slower mineralization of these metabolites in aerobic environments.

The results of the anaerobic tests reveal that, provided organic matter is present, Tioctilate is metabolized fairly rapidly by the bacteria. In oligotrophic anaerobic environments, however, the biodegradation proceeds rather poorly. These results indicate that in the most important anaerobic habitats such as septic tanks, methane digesters, sewers and sludge-waters interphases of rivers, a fairly rapid disappearance of Tioctilate will occur.

1 Seresci, 46, av. Jean-Jaurès, 1030 Bruxelles.
2 W. Verstraete and E. Van Vaerenbergh, Tijdschr. Becewa *4*, 50 (1977).
3 J.P. Voets, P. Pipyn, P. Van Lancker and W. Verstraete, J. appl. Bact. *40*, 67 (1976).
4 OCDE, Pollution par les détergents, Publications de l'OCDE, 2, rue André-Pascal, Paris 16e, France.
5 American Public Health Association, Standard Methods for the examination of water and wastewater, 13th ed. New York.
6 W.K. Fisher, P. Gerike and W. Holtmann, Water Res. *9*, 1131 (1975).

Conjugated terpenoid ketones: A new group of plant growth regulators[1]

P.S. Kalsi, B.R. Chhabra and O.S. Singh

Departments of Chemistry and of Botany, Punjab Agricultural University, Ludhiana (India), 19 July 1978

Summary. α,β-unsaturated terpenoid ketones have a root-inducing property on the hypocotyl cuttings of *Phaseoulus aureus*. Significantly isopatchoulenone (I) is distinctly more active in causeing rooting over IAA.

Terpenoid lactones are emerging as a new group of plant growth regulators. The biological activity of these natural products is associated with the exomethylene group in conjugation with the lactone carbonyl, and this structural feature is almost indispensable[2] for this action. An earlier communication[3] from our laboratory showed that some terpenoid γ-lactones, in which a cyclopropane or a trisubstituted double bond was in conjugation with the lactone carbonyl, are more active than α-methylene-γ-lactones.

Recently[4] terpenoids with a cross conjugated ketone moiety have been shown to cause adventitious root formation in the hypocotyl of mung bean cuttings. In order to obtain