Bull. Environ. Contam. Toxicol. (1994) 53:902–906

Environmental Contamination and Toxicology

Analyses of Liquid Diazinon Formulations and Breakdown Products: An Australia-Wide Survey

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Received: 31 December 1993/Accepted: 2 May 1994

Diazinon (O,O–diethyl–O–[2–isopropyl–4–methyl–6–pyrimidinyl] phosphorothioate) is an organophosphorus insecticide and acaricide with contact, stomach and respiratory action. It is formulated and distributed in a number of forms and is known commercially by several names (Cremlyn 1978; Worthing and Walker 1987). It is effective against a variety of soil, fruit and vegetable pests e.g. aphids, spider mites, thrips and scale insects together with domestic and livestock pests (Eto 1974; Ware 1975).

Diazinon has a fairly low mammalian toxicity LD50 (oral) in rats which ranges from 150 mg to 480 mg per kg (Eto 1974; Worthing and Walker 1983). Under certain conditions diazinon can deteriorate to harmful substances, particularly if the hydrocarbon solvent is contaminated with a small quantity of water (0.1–2%), and exposed to elevated temperatures, air and light (Margot and Gysin 1957). These conditions favour the formation of monothiotepp (O,S–TEPP) and sulfotepp (S,S–TEPP). These compounds are known to be highly toxic and to have a strong inhibitory effect on cholinesterase enzyme systems (Margo and Gysin 1957; Gysin and Margot 1958).

Sovocool *et al.* (1981) and Soliman *et al.* (1982) reported on aged diazinon formulations which had degraded to a number of tetraethylpyrophosphates. Sulfotepp (S,S–TEPP) and monothiono–TEPP (O,S–TEPP) were two of the identified products in the samples. Both compounds are much more toxic than diazinon, especially O,S–TEPP, which showed in one test of enzyme inhibition as being 14,000 times more toxic than diazinon (Soliman *et al.* 1982; Singmaster 1990). In the presence of large amounts of water diazinon hydrolyses to products which are practically non–toxic e.g. ethyl thiophosphate. Ruzicka *et al.* (1967) showed this reaction to be a pseudo first order reaction.

Recently, a number of dog deaths have been reported in NSW and Victoria, possibly due to the use of a diazinon based dog wash. In each case the product had passed the expiry date that the manufacturer had put on the label. O,S and S,S–TEPP were detected in several of the formulations, in one case at levels of 6,600 mg/L and 1,600 mg/L respectively, and were responsible for the mortalities. Whilst in some cases, the animal handlers experienced nausea, fatigue, blurred vision etc. symptoms typical of cholinesterase–inhibiting organophosphate pesticides.

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This work forms part of an extensive screening program conducted throughout the States of Australia, which set out to identify if a problem existed with diazinon formulations containing toxic levels of breakdown products and if so, to ensure they did not find their way into the market place.

MATERIALS AND METHODS

All solvents and reagents used were analytical grade or equivalent. Diazinon (99.5%) and the major breakdown products : O,S–sulfotepp (O,S–TEPP or monothionotepp) and S,S–sulfotepp (S,S–TEPP or sulfotepp) were obtained from Ciba–Geigy Australia Limited (Sydney, Australia). Standard stock solutions (100 μg/mL) were prepared in n–hexane and kept refrigerated.

Logistically, it was not possible to test all of the diazinon formulations available and so, a number of the more popular diazinon products were identified for testing. These included ectoparasiticides for the treatment of sheep, and other food producing species and companion animals, horticultural and house and garden insecticides. Various retail outlets within each State were selected at random and a range of diazinon formulations were sampled by regional pesticide inspectors in the period between May 1993 to June 1993. For container sizes less than or equal to 250 mL volume, the whole container was obtained. However, for very large volumes such as sheep dip preparations (5 litres and greater) a sample of the formulation was drawn off with a syringe and placed into a screw top glass jar or tube. The best sample containers proved to be screw top glass tubes (with teflon seal lid insert) of about 50 mL capacity (to minimise air space above the sample). The samples were then transported to the laboratory (packed in an absorbent packing such as vermiculite or shredded paper) for analysis. Samples were stored at room temperature and analysed within 48 hours of receival.

All liquid formulation samples were initially dissolved in acetone and sample aliquots subsequently diluted with hexane to appropriate volumes, depending upon concentration (these ranged from 1 g/L to 800 g/L). Diazinon and its toxic breakdown products O,S–TEPP and S,S–TEPP, were determined by gas–liquid chromatography using a Varian Aerograph model 3700 gas chromatograph equipped with thermionic specific detection (TSD) and a DB–17 megabore column 30 metres by 0.53 mm id (J&W). Injector, column and detector temperatures were set at 220°, 170° and 320°C respectively. Nitrogen was used as carrier gas at a flow rate of 20 mL/min. Hydrogen and air flows were 1.5 mL/min and 50 mL/min respectively. The retention times for O,S–TEPP, S,S–TEPP and diazinon under the described conditions were 4.2, 4.8 and 6.8 minutes respectively. As rapid TEPP formation takes place in diazinon in the presence of a small quantity of water (Margot and Gysin 1957), any sample which was found to contain O,S–TEPP and S,S–TEPP was subsequently analysed by the Karl–Fischer method for water content (Vogel, 1962).

Table 1. Analyses of diazinon formulations found to contain both O,S and S,S-TEPP.

Product	Origin	Label Claim Diazinon g/L	Water Content mg/mL	O,S-TEPP	S,S-TEPP	Diazinon
				g/L		
Diazinon Dog Wash	NSW	100	0.5	0.14	0.27	97.2
Diazinon Dog Wash	NSW	150	0.7	1.55	0.42	94.7
Sheepdip Blowfly Suppressant	QLD	96	2.2	4.67	<0.03	24.5
Sheepdip Blowfly Suppressant	QLD	96	3.4	5.49	0.48	21.0
Diazinon Stock Spray	NSW	200	3.9	1.24	0.63	195.0
Diazinon Insect Killer	QLD	200	1.8	19.4	3.70	150.0
Diazinon Insect Killer	QLD	200	1.7	18.75	3.95	113.0
Diazinon Insect Killer	QLD	200	6.4	13.4	6.90	135.0

Confirmation of the major breakdown products was carried out on a Hewlett-Packard 5970 MSD-5890 GC system as described earlier (Allender and Keegan 1991).

RESULTS AND DISCUSSION

All seven States of Australia participated in the survey which was coordinated by the National Registration Authority in Canberra, Australian Capital Territory. A total of 169 liquid diazinon formulations representing 23 more commonly purchased products, ranging from dog washes to sheep dips, were tested for the presence of O,S-TEPP and S,S-TEPP, along with a check on diazinon content. Some 26 samples (or 15.4%) of the total sampled, failed to meet the ±10% label claim. However, only eight (8) (or 4.7% of total), of these samples, three from New South Wales and five from Queensland, was it necessary to determine water content by the Karl Fischer method. These samples contained both O,S-TEPP and S,S-TEPP from 0.14 to 19.4 < 0.03 to 6.9 grams per litre respectively. Table 1 lists the analyses of these eight diazinon formulations which had been sampled during the survey. In every sample, water was found to be present, which ranged from 0.5 mg/mL (0.05% w/v) to 6.4 mg/mL (0.64% w/v). It is known that rapid TEPP formation takes place in preparations containing diazinon in the presence of a small amount of water and insufficient content of stabiliser.

Increased toxicity of diazinon (and other phosphorothioate pesticides) may be initiated by certain containers. For example, one type of semilucent plastic bottle appeared to have a much faster degradation of diazinon formulation than other plastic and metal containers (Singmaster 1991 *personal communication*). The latter diazinon preparation, which contained the highest amount of water, was also in a plastic bottle which showed signs of deterioration and may have contributed to the degradation - possibly by absorption of the stabiliser. Further research in this area needs to be conducted by manufacturers of products containing diazinon to ensure the stability and safety of these products.

Acknowledgements

This survey was supported by the National Registration Authority, Canberra and coordinated by Stephen MacDonald. Our thanks to Kerry Smith and Nazir Ahmad for reviewing the manuscript and for their suggestions.

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