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MEMORANDUM

SUBJECT: Diquat: Correspondence from Region III (EPA) Regarding the Teratogenicity and Mutagenicity of Diquat, and the Reinstatement of the 14-day Ban on Swimming in Diquat-Treated Water.

TO: Richard F. Mountfort, Product Manager #23
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On June 14, 1983 EPA approved Chevron's deletion from the label of a warning advising against swimming for 14 days in water sprayed with diquat. This action was recommended by Registration Division (RD) and was based on the following criteria:

1. The upper limit of diquat application for algae control in lakes, ponds and other waters is 1.5 ppm (calculated as the cation). The initial concentration of diquat in water begins to decrease immediately after application and will not, in all probability, cause adverse acute effects in swimmers.

2. "Available summaries of data regarding acute effects show that 400 mg/kg produced no ill effects (Acute Rabbit Dermal study using a technical dibromide, percentage not specified) and an LD50 of 20 - 40 mg cation/kg based on a Subacute Rabbit Dermal study of 20 days duration." (Memorandum from R. F. Mountfort to J. W. Akerman; 6/2/83.)

According to Dr. Bruce Molholt, Region III (EPA), the removal of the 14-day ban on swimming in diquat-treated water poses hazard to humans because diquat has both mutagenic and teratogenic properties. Dr. Molholt, therefore, requested that the 14-day ban be restored and summarized briefly the following studies in support of his concerns:

1. Diquat stimulated unscheduled DNA synthesis in human cells. The incorporation of ^{3}H-thymidine (grains/nucleus) was used as an indication of unscheduled DNA snythesis. (In vitro study conducted in Rome, Italy; Reference 1).

2. Diquat stimulated unscheduled DNA synthesis in human cells transformed by SV40 virus. (Dominant lethal study with CD1 mice; Reference 2).

3. Diquat was both teratogenic and clastogenic in mice which were injected i.p. once or 4x with Reglon, a formulation containing 20% of diquat. Single injections contained 11 mg of Reglon (2.2 mg of diquat)/kg and multiple injections contained 2.7 mg of Reglon (0.54 mg of diquat)/kg. (Reference 3).

References submitted by Dr. Molholt:

1. Benigni, R., M. Bignami, A. Carere, G. Conti, L. Conti, R. Crebelli, E. Dogliotti, G. Gualandi, A. Novelletto and V.A. Ortali. Mutational studies with diquat and paraquat in vitro. Mutation Research 68, 183-193 (1979).

2. Anderson, D., D.R. McGregor and I.F.H. Purchase. Dominant lethal studies with diquat and paraquat in male CD-1 mice. Mutation Research 40, 349-358 (1976).

3. Selypes, A., L. Nagymajtenyi and G. Berencsi. Mutagenic and embryotoxic effects of paraquat and diquat. Bulletin of Environmental Contamination and Toxicology 25, 513-517 (1980).

Toxicology Branch/HED was asked by RD to "review materials/concern for diquat genotoxic potential in treated water submitted by Dr. Molholt, Region III." Because studies were not submitted for the review and brief summaries cannot be substituted for studies, Toxicology Branch will reply to Dr. Molholt's concerns regarding mutagenicity and teratogenicity of diquat.

1. Mutagenicity of Diquat:

Toxicology Branch has 2 studies concerned with mutagenicity of diquat. In one study, dated 7/5/78, Wistar-derived male rats received, by gavage, diquat dibromide monohydrate (100% pure) at the following dose levels: 0, 4.4, 9.5 and 14.0 mg of diquat cation/kg of body weight. There was no chromosomal damage, even at the highest level tested, after 5 consecutive days of treatment of the rats with diquat. Chromosomal damage was assayed in bone marrow cells. Ethyl methanesulphonate (EMS) was used as a positive control.

In another study, dated 2/20/74, diquat, fed orally to Charles River CD1 male mice for 5 consecutive days, did not produce dominant lethal effects at any of the levels tested (0, 0.1, 1.0 and 10.0 mg of diquat cation/kg of body weight). The treated male mice were mated with different untreated female mice each week for a total of 8 weeks. Positive controls (cyclophosphamide and EMS) each produced dominant lethal effects. The test material was a diquat formulation containing 28.6% of diquat cation.

In order to assess mutagenic properties of a pesticide, a battery of mutagenicity tests must be conducted. Presently available data, including Dr. Molholt's comments, are inadequate to regard diquat definitely as a mutagen. It was already indicated to the registrant that additional mutagenic studies will be required. These data will probably be submitted for the Registration Standard Review which, apparently, will soon take place.

2. Teratogenicity of Diquat:

Toxicology Branch has 3 teratology studies with diquat, as follows:

Species Tested	Date of study	Nonteratogenic levels
Rat	6/73	500 ppm (25 mg) of technical grade diquat dibromide/kg body weight.
Rabbit	7/74	5 mg of diquat cation/kg of body weight, administered as 100% pure diquat dibromide monohydrate.
Mouse	7/78	1.0 mg of diquat cation/kg of body weight, administered as diquat dibromide monohydrate (Anal. Std.).

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In all instances, diquat was administered orally, which is one of the common routes of exposure, in the case of diquat. Dermal and inhalation exposures are other routes, especially in the agricultural use of diquat. In cases of water treatment with diquat, to control weeds, dermal exposure will probably be of greatest concern. Teratogenic studies involving i.p. injections contribute to knowledge of the toxicity of diquat, but do not represent a realistic route of exposure.

As far as acute dermal effects are concerned, LD50 = 50-100 mg of diquat cation/kg body weight was reported in one study, dated 7/74, with female SPF rats. Piloerection, depression and weight loss were also observed at the 100 mg/kg level. Diquat dichloride and diquat dibromide (purity not stated) were tested in these studies.

3. Deletion of the 14-Day Swimming Restriction:

Toxicology Branch has never recommended the removal of this restriction. (Please see RD Attachment V, dated May 24, 1983.) Since, to our knowledge, additional data were not submitted by the registrant, Toxicology Branch position regarding this matter remains unchanged.

Comment Regarding R. F. Mountfort's Memo to J. W. Ackerman, Dated June 2, 1983.

It is quoted on page 2 of that memo that, in a subacute dermal rabbit study of 20-day duration, an LD50 of 20-40 mg diquat cation/kg was reported. Unless these data are available to RD, they are not to Toxicology Branch. According to Toxicology Branch data, a No Observable Effect Level (NOEL) and a Least Effect Level (LEL) were determined in a 20-day dermal study with rabbits. In that study, dated 8/66, NOEL was 20 mg/kg and LEL was 40 mg/kg, both expressed as diquat cation.