DATA EVALUATION RECORD

(1) CHEMICAL: Trichlorfon

(2) TYPE OF FORMULATION: Wettable powder (Dipterex)

(3) CITATION: Kadin, M. 1962. Studies on the toxicity and effect of Dipterex in rabbits. Am. J. Opthalmol. 53:512-517

(4) REVIEWED BY:

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(32B-0035)

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(6) TOPIC: This study has information pertinent to discipline toxicology, topic acute intraperitoneal toxicity and biochemistry. It relates to none of the Proposed Guidelines data requirements.

(7) CONCLUSION:

(a) Experiment A: Groups of three albino rabbits were given a single intraperitoneal injection of Dipterex at levels ranging from 50 to 300 mg/kg bw.

Typical signs of cholinesterase inhibition (lacrimation, miosis, salivation, twitching, diarrhea, and convulsions) were observed. For the one animal that survived a dose of 50 mg/kg, these symptoms disappeared after approximately 3 hours. Doses of 100 mg/kg bw and above killed 100% of the animals. Insufficient details were provided to allow calculation of an LD_{50}.

(b) Experiment B: A single drop (approximately 5.0 mg of Dipterex) was placed into the eyes of an unspecified number of rabbits. Maximal inhibition of cholinesterase activity was observed 0.5 hours later in the brain ciliary body and red blood cells (RBC). Serum levels were maximally inhibited at the end of 1 hour. At the end of 2 hours, brain, ciliary body, and serum levels were approaching pretreatment levels. RBC levels remained depressed. Because of uncertainties over dosage and baseline levels, no quantification of these effects is possible.

(c) Experiment C: An unspecified number of rabbits were given Dipterex eye drops in both eyes three times a day for 21 days. Cholinesterase levels in the brain (cortex), serum, red blood cells, and ciliary bodies steadily declined

over the 3-week period. As with Experiment B, conclusions on the quantification of these effects is not possible.

(d) Experiment D: Three rabbits were given an intravenous injection of 5.0 mg/kg bw of Dipterex (10% aqueous solution). An insufficient amount of data was provided to evaluate this study.

CORE CLASSIFICATION: Not applicable

(8) MATERIALS AND METHODS:

Test Substance: The test material was described as Dipterex, a crystalline powder soluble in water and organic solvents. The powder used in this study, (obtained from Drs. Dubois and Doull) was 98.9% pure. It was used in a 10% aqueous solution. No further details were provided.

Organism: Albino rabbits weighing between 2.0 and 3.0 kg were used in all studies. No further details were provided.

(a) Experiment A:

Experimental Procedure: Groups (number unspecified) of rabbits were each given a single intraperitoneal injection of Dipterex ranging from 50-300 mg/kg bw.

Variables Observed: The number of animals dying and the time of death were recorded. Symptoms of poisoning were also observed.

Statistical Methods: The method used to calculate the LD_{50} was not described.

(b) Experiment B:

Experimental Procedure: A single drop of Dipterex in 10% aqueous solution was placed in the eyes of an unspecified number of animals. The animals were observed for 2 hours.

Variables Observed: Cholinesterase measurements were made 30, 60, and 120 minutes after instillation of Dipterex. Cholinesterase levels in the following tissues were measured manometrically using the method of Dubois and Mangun: brain, cortex, serum, red blood cells, ciliary body, and aqueous humor. Total protein determinations in the primary aqueous humor were made by the Nessler method.

(c) Experiment C:

Experimental Procedure: An unspecified number of animals were given Dipterex eye drops (each drop contained approximately 5.0 mg) in both eyes three times a day for 21 days. It was not stated how these three administrations were spaced during the day. Daily dosages were approximately 30 mg/kg body weight. A special effort was made to place drops on the cornea.

Variables Observed: Cholinesterase activity in the brain cortex, ciliary body, serum, and RBC were measured 20 hours after the last instillation.

(d) Experiment D:

Experimental Procedure: Three rabbits were given an intravenous injection of 5.0 mg/kg bw of Dipterex and cholinesterase

activity was measured (tissues and time of measurements were not specified).

(9) REPORTED RESULTS:

(a) Experiment A: The LD_{50} for the intraperitoneal injection of Dipterex for rabbits was found to be 60 mg/kg. Doses of 100 mg/kg and above killed all animals; one of three animals at the 50 mg/kg bw level survived. Survival times ranged from 20 to 50 minutes.

Toxic effects commonly associated with cholinesterase inhibition, i.e., lacrimation, salivation, twitching, diarrhea, and convulsions, were observed. For that animal that survived the dose of 50 mg/kg, these symptoms disappeared within about 3 hours.

Marked miosis was observed in all animals; among the survivors, miosis decreased in parallel with other symptoms.

(b) Experiment B: After the instillation of Dipterex, cholinesterase activity in the brain, ciliary body, RBC, and serum, was decreased. For the first three tissues, maximal inhibition occurred after 30 minutes; serum levels were maximally inhibited after 60 minutes. Cholinesterase activity returned to near normal levels for nerve tissues, but not for blood, in 2 hours (see table below). During the observation period, pupils were markedly constricted to 2 mm. No information on total protein levels was provided.

	Cholinesterase Activity (Percentage of normal activity)		
	30 min.	60 min.	120 min.
Brain (cortex)	48	72	84
Serum	60	58.5	70
RBC	38	50	50
Ciliary body	26.9	35	80

(c) Experiment C: Cholinesterase activity (expressed as a percentage of normal levels) fell progressively over the 3-week period (see table below).

	7 Days	14 Days	21 Days
Brain (Cortex)	84	75	58
Serum	90	75	35
RBC	54	50	37
Ciliary body	45	43	30

The authors also noted that the pupils were 7-8 mm. In the acute studies, similar levels of cholinesterase inhibition were associated with pupils constricted to 2 mm.

(d) Experiment D: The authors reported that intravenous injection of 5 mg/kg bw of Dipterex gave the same range of cholinesterase inhibition as that observed after the use of eye drops.

(10) DISCUSSION: With respect to the observation that administrations of Dipterex depress cholinesterase levels, the results from these four studies are consistent with those reported

elsewhere. However, a number of shortcomings in the study designs and the limited reporting of results makes it impossible to relate these effects to a particular dose level. These shortcomings are enumerated below.

There were several problems with the acute intraperitoneal study. These include the following: (1) The actual dose levels used were not specified, (2) the number of animals per dose level was not specified, (3) no indication of individual survival time was provided, and (4) the method used for calculating the LD_{50} was not specified.

The acute and chronic ocular studies were also inadequately reported. No baseline levels of cholinesterase activity were reported. It is unclear what "normal" levels actually represented. This problem was compounded by the fact that results were only presented graphically. In addition, no details were given on the number of animals tested. The dose levels were also inadequately defined.

In the study involving intravenous injection of Dipterex, the details provided were not sufficient to make any evaluation.

Despite these inadequacies, there are some qualitative conclusions that can be drawn from these studies. First, Dipterex appears to be systemically distributed when placed into the eye, and secondly, Dipterex does cause cholinesterase inhibition and, at high doses, produces signs and symptoms typical of such inhibition.

It is also interesting to note the discrepancy in the relationship between cholinesterase inhibition and constriction of pupils between acute and chronic studies. This may be caused by the animals developing a tolerance to the pupil-constricting effects of Dipterex when it is administered over several weeks. Again, uncertainties over the study design must be considered when interpreting these findings.

(11) TECHNICAL REVIEW TIME: 5.0 hours