Reiner

DATA EVALUATION RECORD

(1) CHEMICAL: Trichlorfon

(2) TYPE OF FORMULATION: Unspecified

(3) CITATION: Reiner, E., and Plestina, R. 1979. Regeneration of cholinesterase activities in humans and rats after inhibition by O,O-dimethyl-2,2-dichlorovinyl phosphate. Toxicol. Appl. Pharmacol. 49:451-454

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

(7) CONCLUSION: Regeneration in vivo of rat brain cholinesterase activities, following inactivation with doses of trichlorfon or DDVP, can be attributed to spontaneous reactivation of the inhibited enzyme.

Regeneration in vivo of rat plasma and human plasma cholinesterase activities, following inactivation with doses of trichlorfon, can be attributed to spontaneous reactivation of the inhibited enzyme and/or to synthesis of new enzyme molecules.

Regeneration in vivo of human erythrocyte cholinesterase proceeds slowly and may be associated with synthesis of new enzyme.

CORE CLASSIFICATION: Not applicable

(8) MATERIALS AND METHODS: Trichlorfon was obtained from Bayer Farbenfabriken (Wuppertal, West Germany) and DDVP from the World Health Organization (Geneva, Switzerland). The purity of the compounds was unspecified. Solutions were prepared in glycerinformal (75% 5-hydroxy-1,3-dioxan plus 25% 4-hydroxymethyl-1,3-dioxolan). Trichlorfon (300 mg/kg) and DDVP (2.5 mg/kg) were injected intravenously into male albino rats (strain not specified) weighing about 300 g.

At suitable time intervals, the rats were killed, blood was taken from the heart, and brains were removed. Plasma was prepared by centrifugation, and brains were homogenized (40 mg/ml) in ice-cold 0.15 M NaCl. Cholinesterase activities in plasma and brain were measured spectrophotometrically by the method of Ellman et al. (1961. Biochem. Pharmacol. 7:88) with acetylthiocholine as substrate. Details of the procedures were described previously (Skriwjaric-Spoljar et al. 1973. Biochim. Biophys. Acta 315:363).

The data derived for rats were compared to those previously derived for humans and published (Plestina et al. 1972. Bull. WHO 46:747). The enzyme assay for erythrocyte and plasma cholinesterases was the same as that described above. For the previous report, data were obtained from three groups of children given oral doses of 7.5, 10, and 12.5 mg/kg of trichlorfon.

(9) REPORTED RESULTS: Brain and plasma cholinesterase activities for rats injected with trichlorfon or DDVP are in the following table:

Time After Dosing	Cholinesterase Activity (%)[a]			
	Trichlorfon		DDVP	
	Brain	Plasma	Brain	Plasma
0.5 hr	2	7	15	41
1 hr	6	7	30	44
1.5 hr	13	14	36	59
2 hr	14	19	50	74
3 hr	35	33	56	93
6 hr	68	73	85	94
12 hr	68	74	83	100
24 hr	84	95	87	103
2 days	78	93	90	
6 days	83	95	82	
8 days	77	102	82	

[a]Expressed as % activity of 40 untreated rats. Each value is the mean obtained for 5-12 rats.

Erythrocyte and plasma cholinesterase activities for humans (school children) treated orally with 7.5 (group A), 10 (group B), or 12.5 (group C) mg/kg of trichlorfon are in the following table:

Days After Dosing	Cholinesterase Activity (%)[a]					
	Group A		Group B		Group C	
	Erythrocyte	Plasma	Erythrocyte	Plasma	Erythrocyte	Plasma
0.25	78	5	43	0	59	0
1	87	19	51	17	60	14
3	72	28	63	35	76	31
7	80	64	65	47	62	56
14	92	76	72	71	80	72

[a]Activities are expressed as % of activity before treatment. Each number is the mean value for groups A, B, and C, which contained 21, 19, and 19 individuals, respectively.

The calculations made were based on these data. The highest degree of enzyme inhibition was considered to be "100% inhibition," and the degrees of inhibition at other times were adjusted accordingly. The time course of regeneration of enzyme activity was determined by plotting "log % inhibition" vs time after dosing. For both humans and rats, mean values were used for points in these plots. For humans, the mean values of groups A + B + C were used.

On the assumption that restoration of cholinesterase activity was due only to spontaneous reactivation of the inhibited enzymes, a theoretical regeneration curve was

calculated based on the equation (Skrinjaric-Spoljar et al., Biochim. Biophys. Acta 315, 363, 1973):

$$100 - \% \text{ inhibition} = \frac{100\ k_r}{k_r + k_{ag}}\ 1 - e^{-(k_r + k_{ag})r}$$

where k_r and k_{ag} are rate constants for spontaneous reactivation and aging of the enzyme inhibited by DDVP and t is the time of the reaction. Rate constants (not stated) previously determined at 37°C and pH 7.4 were used.

For rats, there was good agreement between the theoretical and experimental results. According to the theoretical curve, 90% of the plasma cholinesterase and 80% of the brain cholinsterase should reactivate in 10 hr; the remaining enzyme should rmain inhibited indefinitely.

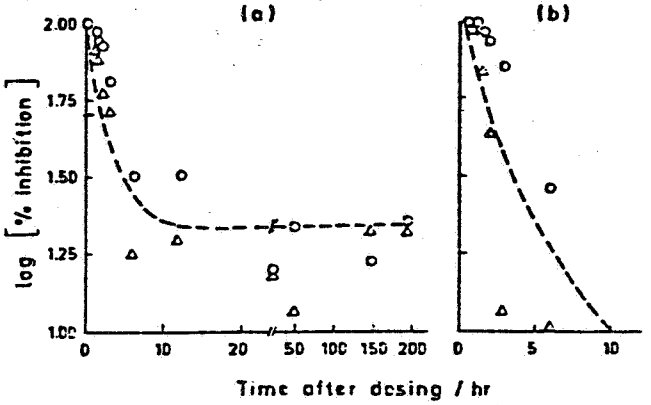

Figure 1. Regeneration of cholinesterase activity in brain (a) and plasma (b) of rats dosed iv with DDVP (Δ) or trichlorfon (O). The points are calculated from resutls in Table 1. The dotted line is a theoretical line.

The data derived for the human enzymes are different from the theoretical values. There are linear (first-order) plots for both erythrocyte and plasma cholinesterases; the calculated half-times for regeneration of activity are 15 and 6.7 days, respectively. The theoretical calculations for the erythrocyte and plasma enzymes, however, predict half-times of 57 min and 3.4 days (or less) respectively. The discrepancies suggest that restoration of cholinesterase activity in both erythrocytes and plasma is due, at least in part, to enzyme synthesis.

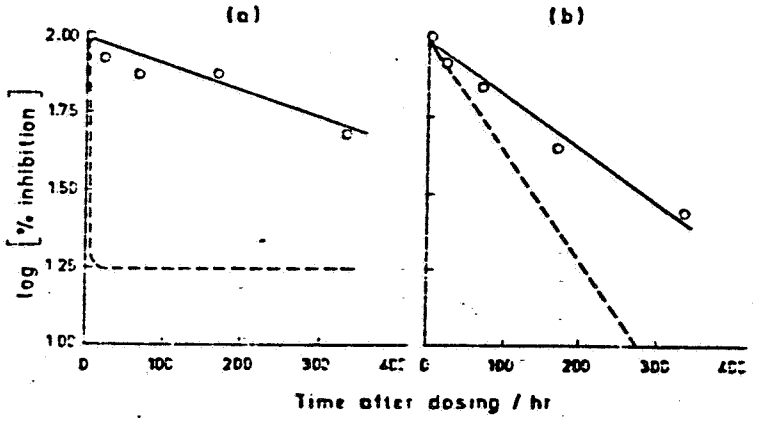

Figure 2. Regeneration of cholinesterase activity in erythrocytes (a) and plasma (b) of school children treated orally with trichlorfon. The points are calculated from results in Table 2 and are mean values obtained in groups A, B, and C. The dotted line is a theoretical line.

(10) DISCUSSION: In this manuscript, rate constants, previously determined for spontaneous reactivation and aging of various mammalian cholinesterases in in vitro experiments, are applied to enzyme activities measured in vivo. Since the rate constants used for calculating the theoretical curves were not included in the present manuscript, the reliability of the calculations for these values can not be assessed.

The calculated half-time for recovery of the human erythrocyte enzyme (15 days) is said to be comparable to the reported half-time for synthesis of this enzyme (Grob et al., Bull. Johns Hopkins Hosp. 81, 217, 1947). The actual half-time may be even larger, for none of the points is significantly different from the others. For example, the first point is 60 ± 18% and the last point is 81 ± 10% (mean ± std dev). In any case, however, there is a great difference between the theoretical and derived values.

The value of 6.7 days for recovery of the human plasma enzyme is said to be comparable to reported half-times for enzyme synthesis of 7.5 (Grob et al., Bull. Johns Hopkins Hosp. 81, 217, 1949), 11-12 (Neitlich, J. Clin. Invest. 45, 380, 1966), and 14 days (Boyer et al., Toxicol. Appl. Pharmacol. 41, 389, 1977).

The theoretical curve for regeneration of cholinesterase activity in the rat brain fits well with the derived points,

providing evidence that enzyme regeneration occurs in this organ. Although the authors state, for the plasma enzyme, that the theoretical curve fits the derived points, this is not the case if one considers only the data for trichlorfon. For this agent, the half-time for recovery of enzyme activity is about 5 hr, twice the theoretical value. (For the human plasma enzyme, a half-time twice as great as the theoretical value was considered to be substantially different.)

The following factors may have contributed to the observed differences in rat and human cholinesterase activity regeneration but are not considered: (1) dosage level, (2) route of administration, (3) species, (4) age.

The authors do not speculate on why the human erythrocyte enzyme could undergo spontaneous restoration in vitro but not in vivo.

(11) TECHNICAL REVIEW TIME: 9.0 hours