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Chlorpyrifos-induced delayed polyneuropathy*

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Abstract. Chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-pyridyl) phosphorothioate] caused delayed polyneuropathy in man. Contrary to previous studies, we report here that it also causes delayed polyneuropathy in the hen, the animal model for this toxicity. The minimal neuropathic dose was 60–90 mg/kg p.o., corresponding to 4–6 times the estimated LD_{50}. Consequently, pralidoxime (2-PAM) in conjunction with atropine was necessary to reverse acetylcholinesterase (AChE) inhibition and cholinergic toxicity in hens given high enough doses of chlorpyrifos to cause neuropathy. Chlorpyrifos was slowly absorbed after single oral doses and the threshold of inhibition (>70%) of neuropathy target esterase (NTE), the putative target for delayed neuropathy, was reached within 5–6 days. High AChE inhibition (>90%), however, was measured within hours after dosing because of the higher potency of chlorpyrifos to inhibit this enzyme. In vitro studies showed that chlorpyrifos-oxon, the active metabolite of chlorpyrifos, was 10–20 times more active against AChE than against NTE, confirming the clinical observation. No differences were seen between human and hen enzymes in this respect. Hen and human brain homogenates contain A-esterases which hydrolysed chlorpyrifos to about the same extent in both species. In conclusion, chlorpyrifos causes delayed polyneuropathy in the hen, as was reported in man. The reasons for previous negative data in the hen are probably due to the relatively lower doses which were used. Judging from in vitro studies with hen and human enzymes, there are no differences in the two species as far as their relative sensitivity to delayed polyneuropathy. It is likely that delayed polyneuropathy would develop in both species only after severe cholinergic toxicity requiring aggressive antidotal treatment.

Key words: Chlorpyrifos – Acetylcholinesterase – Neuropathy target esterase – A-Esterases – Organophosphates – Polyneuropathy

Introduction

Chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is a broad range organophosphorus (OP) insecticide (Worthing 1987), widely used in agriculture and indoor disinfestation.

Chlorpyrifos toxicities are caused by its activated oxygen analog chlorpyrifos-oxon, which is a more active inhibitor of esterases. Activation occurs mainly in the liver, but it is likely to be more important in other organs (Sultatos 1988). Experiments using mouse liver perfusion techniques indicated that chlorpyrifos-oxon was produced and also hydrolyzed very efficiently by liver enzymes, preventing the passage of intrahepatically-generated chlorpyrifos-oxon into the general circulation (Sultatos et al. 1984). OP hydrolysis by enzymes called A-esterases is known to occur in several other organs including plasma (Aldridge and Reiner 1972).

Accidental ingestion of chlorpyrifos causes acute toxicity in man (Lores et al. 1978), which is known to be due to inhibition of acetylcholinesterase (AChE) at nerve endings and to the subsequent accumulation of acetylcholine. Occupational exposures after indoor spraying led to acute symptomatology in five office workers (Hodgson et al. 1986), whereas no cases of acute toxicity have been described in other studies on workers exposed during chlorpyrifos manufacturing (Brenner et al. 1989) and in agriculture (Hayes 1982). There is considerable variation in the susceptibility of different species to the cholinergic effects of chlorpyrifos (McCollister et al. 1974).

Organophosphate-induced delayed polyneuropathy (OPIDP) is a rare non-cholinergic toxicity caused by some OPs only and was reported in man after poisoning with

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several OP pesticides (Lotti et al. 1984; Lotti 1987). OPIDP is related to the inhibition of an enzyme in the nervous tissue called neuropathy target esterase (NTE). The phosphorylation of NTE and the subsequent molecular rearrangement of the phosphorylated NTE, called aging, are thought to initiate OPIDP (Johnson 1982). When such changes affect more than 70% NTE, usually within hours after dosing, they correlate with the clinical onset of OPIDP 2–3 weeks later. The potential of OP pesticides to cause OPIDP is routinely tested in the hen (OECD 1983; Lotti 1990).

All but two OP pesticides which caused OPIDP in man were positive in the hen test. Methamidophos (0,S-dimethyl-phosphoroamidothioate) caused several cases of OPIDP in man, but failed in the hen (Senanayake and Johnson 1982) and a possible explanation of this species selectivity is the relatively lower cholinergic toxicity of one optical isomer of methamidophos in man (Lotti and Caroldi 1990). A case of OPIDP was observed after massive suicidal intoxication with chlorpyrifos (Lotti et al. 1986). This contrasts with previous reports where chlorpyrifos was found negative in the hen test (Miyazaki and Hodgson 1972; FAO/WHO 1973; Dow Chemical Company, private communication). Moreover, when NTE inhibition was measured in hen brain 24 h after dosing, it was below the threshold, suggesting that the clinical response would have been negative 2 weeks later (Johnson 1981).

This paper shows that high doses of chlorpyrifos also cause OPIDP in the hen, and that the reason for the previously negative response is the insufficient dose of chlorpyrifos which was given to the hens. Some peculiar aspects of chlorpyrifos pharmacokinetics in the hen are also reported.

Materials and methods

Chemicals

Chlorpyrifos (99% purity), labelled as "pure" in the text, was a gift of Dow Chemical Co., Midland, MI, USA. Commercial formulations of chlorpyrifos (40% and 60% a.i. in methylene chloride), labelled as "commercial" in the text, were purchased from local dealers. Chlorpyrifos-oxon was a gift of L. G. Costa, University of Washington, Dept of Environmental Health, Seattle, WA, USA. Mipafox (N,N-diisopropyl phosphorodiamidofluoridate) was a gift of M. K. Johnson, MRC Toxicology Unit, Carshalton, UK. Phenyl valerate was purchased from Lark Enterprises, Webster, MA, USA. DFP (di-isopropyl phosphorofluoridate) was purchased from Fluka AG Chem. Fab., Buchs, Switzerland. Paraoxon (diethyl *p*-nitrophenyl phosphate), acetylthiocholine iodide, 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), and physostigmine were purchased from Sigma Chem. Co., St Louis, Mo, USA. Atropine sulphate was purchased from Ega-Chemie, Steinheim, FRG. Pralidoxime methylsulphate (2-PAM) was a gift of Carlo Erba, Milano, Italy. All other chemicals were of highest analytical grade.

Animals and tissue preparation

Adult hens (*gallus-gallus domesticus*, 1.5–2.5 kg body wt) were caged in groups of 10–15 and fed ad libitum. Food was withdrawn 12 h before dosing. Chemicals were dissolved in glycerol formal and given by gavage (p.o. <2.3 ml) or subcutaneously (s.c. <1 ml). Atropine (20 mg/kg) and physostigmine (0.1 mg/kg) dissolved in saline were given intraperitoneally (i.p.) 10 min before treatment with OPs as standard prophylaxis for cholinergic toxicity. Additional treatment with atropine (20 mg/kg) and 2-PAM (100 mg/kg in saline, i.p.) was given when necessary.

Animals were killed by decapitation, brain, spinal cord and sciatic nerve were removed immediately and washed in ice-cold buffer (TRIS/HCl, 50 mM containing 0.2 mM EDTA, pH 8.0). Tissues were immediately homogenized for assays or stored at –80° C until assayed. Birds were observed daily for cholinergic toxicity and OPIDP and the degree of ataxia was assessed according to a 0–4 point scale (Johnson and Barnes 1970).

Human nucleus caudatus and cerebral cortex were obtained during post-mortem examinations performed within 36 h after death, from the bodies of patients who died of extranervous causes. Samples were then subdivided into fragments of about 300 mg, washed and stored at –80° C. Homogenates of nucleus caudatus or homogenates of nucleus caudatus and cortex were used for enzyme assay.

Assays

Human and hen brain NTE activity was assayed according to Johnson (1977). For peripheral nerve NTE assay, a modification of Johnson's method was used (Caroldi and Lotti 1982; Moretto et al. 1989). AChE activity was measured according to the method of Ellman et al. (1961), slightly modified. AChE hydrolysis in spinal cord and peripheral nerves followed the incubation of homogenates with DTNB for 10 and 30 min, respectively, in order to eliminate DTNB reduction due to components other than enzymatically generated thiocholine.

Acute toxicity experiments

a. Four groups of five birds were administered 4, 8, 16 or 32 mg/kg p.o. of "pure" chlorpyrifos and observed for cholinergic symptoms and mortality.
b. Three groups of five birds were administered 4, 8, or 16 mg/kg p.o. of "commercial" chlorpyrifos and observed for cholinergic symptoms and mortality.

Delayed neurotoxicity experiments

Birds were subdivided as follows:

a. Two groups of 21 birds each were dosed with 60 and 120 mg/kg p.o. of "pure" chlorpyrifos after prophylaxis with atropine/physostigmine. Treatment with atropine and oximes was then given twice a day for 6 days. Three to four animals were killed at daily intervals to measure enzyme activities.
b. Thirty-one birds were dosed with 90 mg/kg p.o. of "pure" chlorpyrifos after prophylaxis with atropine/physostigmine. Treatment with atropine and oximes was then given twice a day for 6 days. Birds were then treated once a day for another 5 days and observed for 2 weeks for OPIDP. Three to four animals were also killed at daily intervals to measure enzyme activities.
c. Twenty-six birds were dosed with 150 mg/kg p.o. of "commercial" chlorpyrifos after prophylaxis with atropine/physostigmine. Treatment with atropine/oximes was given twice a day for 5 days, then only once a day for another 3 days. Two animals were killed daily for enzyme assays. Surviving animals were observed daily for a further 2 weeks for OPIDP.
d. Positive controls included 14 birds dosed with DFP (1.5 mg/kg s.c.), which also received atropine/physostigmine prophylaxis. Two birds were killed at daily intervals to measure enzyme activities.

Control animals were given vehicle but not the anticholinergic treatment and either killed at daily intervals or observed clinically. Percentages of enzyme inhibition in experimental birds were calculated from the mean activity of all these controls, unless otherwise stated.

Effect of route of administration on AChE inhibition

Hens were divided into four groups (12 birds each) and dosed as follows:

- Chlorpyrifos ("pure", 9 mg/kg p.o., vol <1 ml)
- Chlorpyrifos ("pure", 9 mg/kg i.v., vol <100 µl)
- Chlorpyrifos-oxon (0.15 mg/kg i.v., vol <100 µl)
- DFP (1 mg/kg s.c., vol <1 ml)

All animals received atropine prophylaxis. Control birds ($n = 6$) received both prophylaxis and vehicle by different routes.

Birds for each experimental group ($n = 3$) were killed 4, 24, 48 and 120 h after poisoning to measure brain AChE activity. Percentage of AChE inhibition was calculated from the activity of controls killed during the experiment.

Kinetics of NTE and AChE inhibition by chlorpyrifos-oxon

Human nucleus caudatus (3.8 mg tissue/ml TRIS) was incubated with 6.5 or 13 nM and hen brain (5.0 mg tissue/ml TRIS) with 6.0 or 9.0 nM chlorpyrifos-oxon. AChE activity was measured at intervals up to 60 min.

Different concentrations of homogenates were also used to compare the time-course of inhibition by chlorpyrifos-oxon of both AChE and NTE in hen brain. Inhibitor 50 nM was incubated with hen brain homogenates (6.6, 10, 20, and 40 mg/ml buffer), and NTE activity was measured at intervals up to 300 min. Similarly, AChE was inhibited with chlorpyrifos-oxon 4 nM and the tissue concentrations were 3.3, 6.6, 10.0, and 20 mg/ml buffer.

Fixed time I_{50}s for chlorpyrifos-oxon have been calculated after 20 min incubation of NTE (tissue concentration 6.6 mg) and AChE (tissue concentration 5.0 mg/ml) at 37° C, at pH 8.0 (TRIS) and pH 7.4 (phosphate buffer), respectively. Inhibitor concentrations ($n = 8$) were in the range 0.05 – 1 µM for NTE and 1 – 50 nM for AChE. I_{50}s for both enzymes in hen brain and nucleus caudatus were then calculated according to Lotti and Johnson (1978).

Reactivation by oximes of AChE inhibited by chlorpyrifos-oxon

Hen brain homogenate (6.6 mg tissue/ml TRIS) was incubated with chlorpyrifos-oxon (150 nM) for 20 min at 37° C. Excess of inhibitor was removed by centrifugation (30000 *g*, 20 min, 4° C, twice). The pellets were resuspended in TRIS and incubated at 37° C with pralidoxime methylsulphate 5 µM. The reappearance of AChE activity was then measured at intervals up to 180 min. K_{+3} of reactivation was calculated from the $t^1/_2$ of enzyme activity reappearance derived from a semilog plot of log % inhibition versus time.

Results

Acute oral toxicity of chlorpyrifos is reported in Table 1, indicating that "pure" and commercial chlorpyrifos are similar in this respect. Doses of chlorpyrifos corresponding to 3 – 4 times the estimated LD_{50} caused OPIDP in the hen, as shown in Table 1. To survive such high doses, atropine alone was not sufficient and treatment with 2-PAM was also necessary (unpublished observation). When tested in vitro on AChE inhibited by the active metabolite chlorpyrifos-oxon, 2-PAM reactivated about 60% of 100% inhibited AChE. This was achieved after about 3 h incubation with pralidoxime and the calculated k_{+3} was 6.9×10^{-3} (min^{-1}). No spontaneous reactivation occurred.

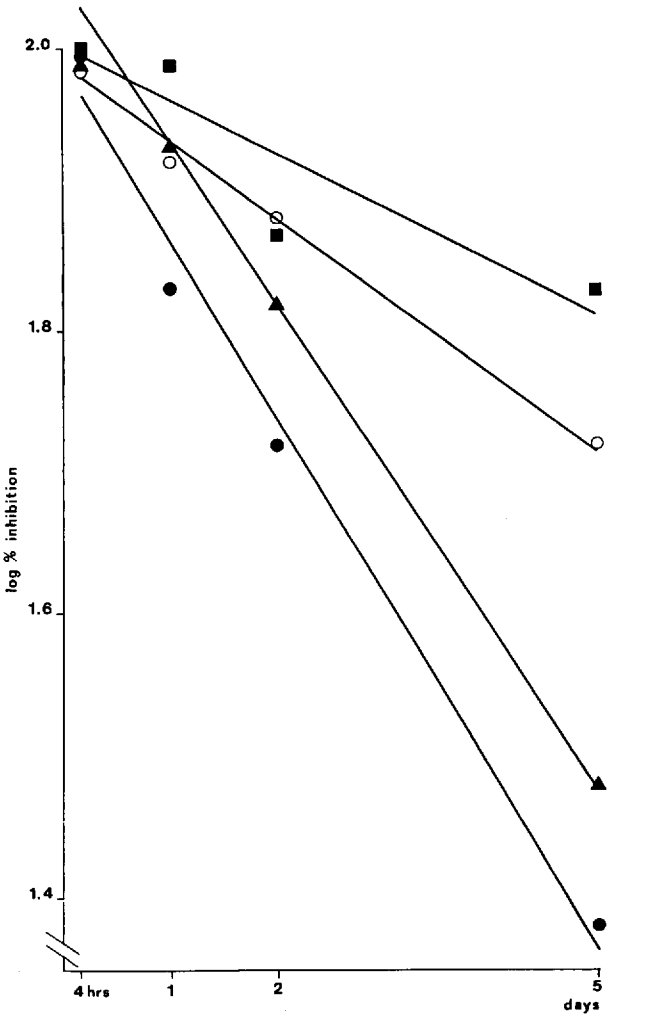

Fig. 1. Reappearance of AChE in hen brain was calculated by regression analysis of all time points in all animals after chlorpyrifos (9 mg/kg i.v. ●——●, $t^1/_2 = 2.4$ $r = 0.78$), chlorpyrifos (9 mg/kg p.o. ■——■, $t^1/_2 = 8.3$ $r = 0.58$), chlorpyrifos-oxon (0.15 mg/kg i.v. ▲——▲, $t^1/_2 = 2.6$, $r = 0.78$) and DFP (1 mg/kg s.c. ○——○, $t^1/_2 = 5.5$ $r = 0.98$). Time points are the mean of experimental data ($n = 3$). AChE inhibition was calculated from six controls whose AChE activity was 2989 ± 96 nmole/min/g of tissue (mean ± SE)

Table 1. Effects of single oral doses of chlorpyrifos in the hen

Dose (mg/kg)	Treatment[a]	Death/survivors[b]	Ataxia score[c] (mean ± SE)
4	–	1/5 (0/5)	0
8	–	1/5 (2/5)	0
16	–	3/5 (2/5)	0
32	–	3/5	0
90	+	3/10	1.1 ± 0.6 (4/7)
150	+	(0/6)	2.5 ± 0.6 (6/6)

[a] Treatment with atropine/2-PAM for cholinergic toxicity as described in the Methods

[b] Deaths occuring within 48 h. The results using "commercial" chlorpyrifos are reported in brackets

[c] Maximal score observed within 25 days after dosing. The number of animals affected/animals dosed is reported in brackets

Table 2. Effects of chlorpyrifos and DFP on nervous tissue NTE and AChE

Chemical	Enzyme	Tissue	Days after dosing										
Dose & route			0.4	1	2	3	4	5	6	7	8	9	11
Chlorpyrifos	NTE[a]	brain		17±7	56±9	33±8	57± 8*	50±2*	61±4*				
60 mg/kg p.o.	AChE[a]			95±0	95±0	88±1	78± 7	75±9	93±1				
90 mg/kg p.o.	NTE[a]	brain		4±1	17±5	17±9	37±11*	47±4*	80±8				
	AChE[a]			73±9	73±1	83±1	82± 7	87±1	80±4				
120 mg/kg p.o.	NTE[a]	brain		17±1	21±6	54±1	59± 1	60±1	59±1				
	AChE[a]			88±4	77±1	85±7	90± 4	81±1	88±4				
150 mg/kg p.o.	NTE[b]	brain	0,10	34, 48	51, 52	53, 86	87, 97	–	77,78	73, 89	63, 89	80,81	55,60
	AChE[b]		52,98	95,100	95,100	100,100	100,100	–	93,70	95,100	79,100	95,95	90,45
	NTE[b]	s.cord	0,18	28, 45	33, 38	46, 75	80, 95	–	71,73	59, 84	53, 83	72,75	44,55
	AChE[b]		37,90	100,100	100, 95	100,100	100,100	–	90,66	100,100	83,100	90,90	90,51
	NTE[b]	p.nerve	0,13	16, 49	47, 69	82, 83	85, 87	–	76,76	70, 90	54, 89	72,74	47,52
	AChE[b]		29,71	65, 71	88, 76	71, 47	76, 76	–	6,59	65, 88	12, 88	88,88	35,82
DFP	NTE[b]	brain	93,96	89, 92	79	70, 67	69, 62	–	55,59	–	–	–	41,40
1.5 mg/kg s.c.	AChE[b]		83,90	74, 80	70	48, 55	50, 50	–	48,41	–	–	–	24,35
	NTE[b]	s.cord	93,90	90, 92	65, 67	66, 70	66, 65	–	63,59	–	–	–	40,36
	AChE[b]		90,80	66, 95	51, 61	87, 36	46, 51	–	32,56	–	–	–	32,41
	NTE[b]	p.nerve	95,96	85, 98	92, 91	82, 75	75, 85	–	30,53	–	–	–	27,12
	AChE[b]		70,76	24, 76	47, 47	0, 18	59, 18	–	12,29	–	–	–	41,18

Data are expressed as percentage inhibition; individual animals or mean ± SE ($n = 3$, except for * where $n = 4$)

[a] Percentage activity was calculated from the mean values (±SE) of all controls ($n = 6$), one killed per day. NTE = 2141±39 nmole/min/g of tissue. AChE = 23.8±0.45 μmole/min/g of tissue

[b] Percentage activity was calculated from the mean values (±SE) of controls ($n = 10$), one killed per day. NTE (nmole/min/g of tissue) was in brain = 2555±90, in spinal cord = 688±31, and in peripheral nerve = 230±17. AChE (μmole/min/g of tissue) was in brain = 26.9±1, in spinal cord = 5.2±0.3, and in peripheral nerve = 0.54±0.04

The time-courses of nervous tissue NTE and AChE inhibition after different single doses of chlorpyrifos are shown in Table 2. AChE was rapidly inhibited at all dose levels and inhibition was maintained for several days. Concurrent experiments in animals treated with doses of DFP above the LD_{50} showed that high inhibition of AChE was also achieved within a few hours after dosing but recovery occurred more rapidly. Scattered data on peripheral nerve AChE inhibition probably reflect a lower precision of the assay method.

After all doses of chlorpyrifos, NTE activity showed increasing inhibition reaching the maximum (corresponding to the threshold for OPIDP initiation) within 5–7 days in all tissues. No significant differences were observed in enzyme inhibition when chlorpyrifos was given at doses between 60 and 150 mg/kg. DFP-treated control birds, however, showed maximal NTE inhibition within hours after dosing and then the reappearance of enzyme activity had a half-life of 5–6 days. No significant differences were detected in NTE inhibition among different nervous tissues, as might occur with other inhibitors (Moretto et al. 1989), suggesting the same access of chlorpyrifos into all nervous system.

These results suggested a slow disposal of chlorpyrifos when given orally to hens. The effect of the route of administration on brain AChE inhibition is shown in Fig. 1. Chlorpyrifos and chlorpyrifos-oxon were given at doses causing, 4 h after dosing, 70–90% of brain AChE inhibition and the reappearance of AChE was determined. When inhibitors were given i.v., however, the speed of AChE reappearance was higher.

Comparative in vitro studies with hen and human enzymes showed that the time-course of chlorpyrifos-oxon inhibition was not first order kinetics (Figs. 2 and 3). Since in vitro diethyl phosphorylated esterases do not reactivate spontaneously (Aldridge and Reiner 1972), this non-linear kinetics is probably due to a reduced inhibitor concentration, because of the presence of high A-esterases. This is shown indirectly in Fig. 3 where kinetics of inhibition of both NTE and AChE from hen brain change by increasing the homogenate concentration. Fixed time I_{50}s, particularly when derived after incubation with highly concentrated homogenates, are therefore a rough estimate of the rate constants of enzyme inhibition. Nevertheless, with a relatively low concentration of tissue in the homogenates (6.6 mg/ml for NTE and 5 mg/ml for AChE) and a short time of incubation (20 min), I_{50}s for chlorpyrifos-oxon were found to be 150 and 180 nM for NTE, 6 and 13 nM for AChE, for hen and human nervous tissue, respectively.

These data suggest that there are no major differences in the sensitivity to chlorpyrifos-oxon of the target enzyme NTE and AChE in both hen and man, nor in the activity of nervous tissue A-esterases hydrolysing chlorpyrifos-oxon.

Discussion

Chlorpyrifos causes OPIDP in the hen and correlates with NTE inhibition above the threshold, confirming similar findings in man (Osterloh et al. 1983; Lotti et al. 1986). No significant differences have been found using either "pure" or "commercial" chlorpyrifos and therefore it is unlikely that differences in toxicological data would arise from biologically relevant impurities present in commercial formulations. The dose required to cause OPIDP in the hen is well above the LD_{50} and our animals needed aggressive

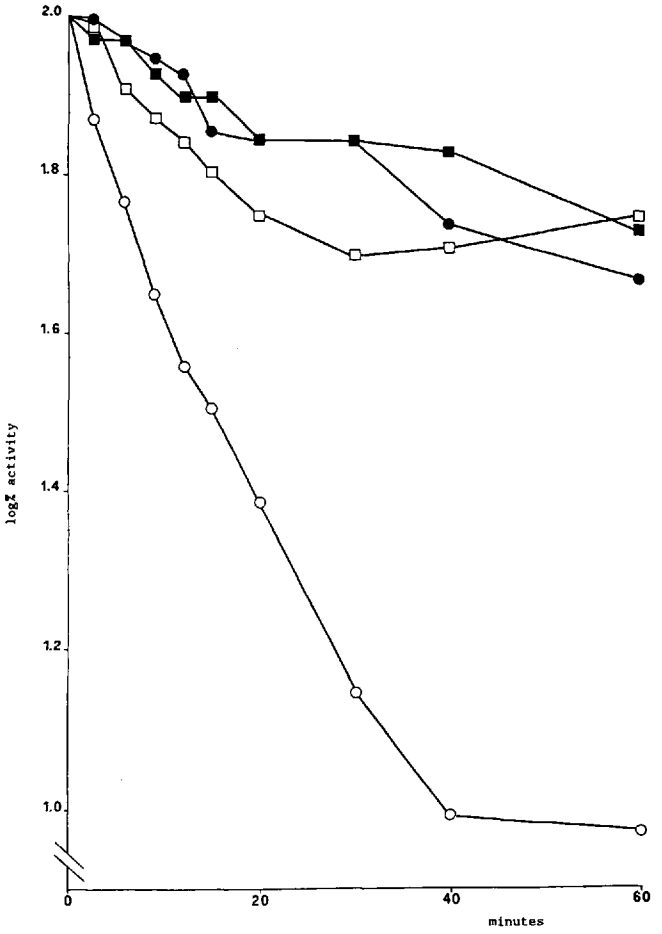

Fig. 2. Time-course of AChE inhibition by chlorpyrifos-oxon. Hen brain homogenates (5.5 mg/ml) were incubated as described in methods with chlorpyrifos-oxon 6 nM (■——■) and 9 nM (●——●); human nucleus caudatus (3.8 mg/ml) with 6.5 nM (□——□) and 13 nM (○——○) chlorpyrifos-oxon

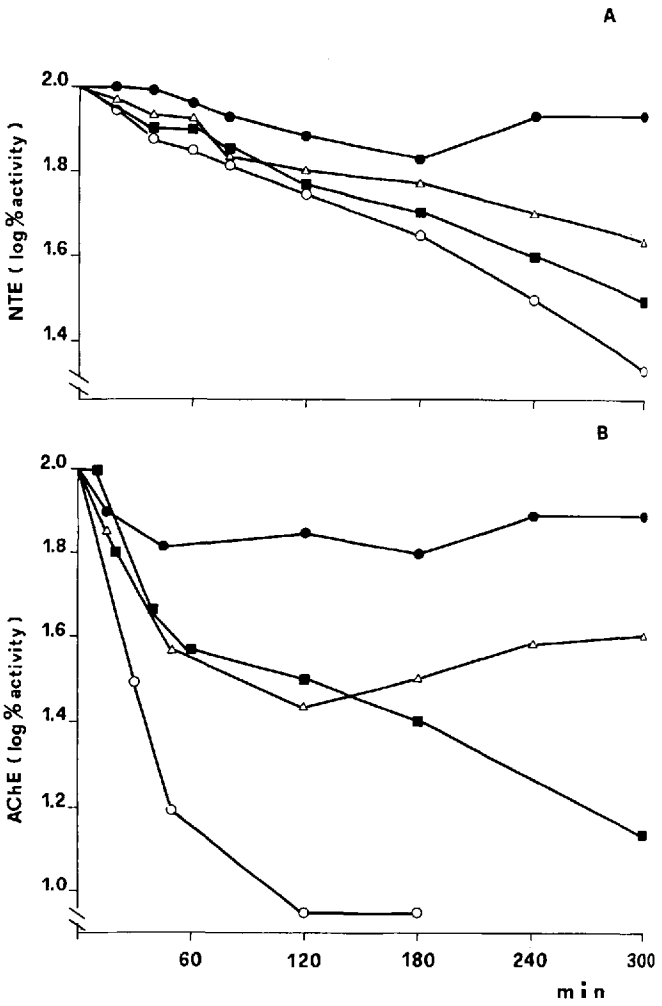

Fig. 3. Effect of tissue concentration of hen brain homogenate on the time-course of NTE (**a**) and AChE (**b**) inhibition by chlorpyrifos-oxon. Incubation conditions and inhibitor concentrations are described in the methods. Tissue concentrations were 6.6 (○——○), 10.0 (■——■), 20.0 (△——△), and 40.0 mg/ml (●——●) in the NTE assay and 3.3, (○——○), 6.6 (■——■), 10.0 (△——△), and 20.0 mg/ml (●——●) in the AChE assay

treatment with atropine/2-PAM for cholinergic toxicity in order to survive and then display signs of ataxia.

A number of reasons might account for the negative results in previous hen studies. For instance, Miyazaky and Hodgson (1972) used chicks of a few days of age in their repeated dosing experiments, and developing birds are known to be resistant to OPIDP (Johnson and Barnes 1970). The study described in the FAO/WHO report (1973) lacks details, whereas that of Johnson (1981) reported measurement of NTE but animals were not observed for OPIDP. Our biochemical data agree with that of Johnson, because he found about 40% NTE inhibition in hen brain, 24 h after dosing with chlorpyrifos (100 mg/kg p.o.). Negative results from Dow Chemical Company (personal communication) were probably due to the relatively high ratio LD_{50}/tested dose, as compared with that used in these experiments. When assessing the potential to cause OPIDP of a given OP, it is always necessary to calculate the ratio LD_{50}/neuropathic dose (Lotti and Johnson 1978), particularly when different LD_{50} values are reported. For instance, our oral acute toxicity data of chlorpyrifos are similar to an oral LD_{50} of 32 mg/kg reported by McCollister et al. (1974), but different from what can be derived from Moore et al. (1989). In fact, extrapolating from brain AChE inhibition their LD_{50}s would be much higher than that calculated in our experiments.

A peculiar pharmacokinetics of chlorpyrifos was observed in these studies. The differences in the speed of reappearance of AChE after inhibition by chlorpyrifos and chlorpyrifos-oxon when given p.o. or i.v. indicate a slow absorption of the compound by the gastrointestinal tract and AChE reinhibition. The half-lives of AChE reappearance after both chlorpyrifos and chlorpyrifos-oxon given i.v. are similar, suggesting that pharmacokinetic differences are unrelated to metabolic activation. Because of this slow pharmacokinetics of chlorpyrifos, we observed a build-up of NTE inhibition, reaching the threshold for OPIDP initiation only several days after single doses. A similar pattern was also observed on AChE inhibition when much lower doses of chlorpyrifos were given orally (unpublished observation). A much shorter delay on NTE inhibition, up to 48 h after dosing, was observed with other OPs (Johnson 1982). In general, however, NTE inhibition

occurs within 24–48 h after single doses of OPs and then NTE activity starts to reappear. The timing of NTE measurement therefore becomes crucial when this biochemical assay is used for OPIDP risk assessment (Lotti 1990), as in the case of chlorpyrifos, the usual assay (24–48 h after dosing) would not correlate with OPIDP resulting a false negative.

Comparative in vitro studies with hen and human enzymes have shown that the two species seem to be equally susceptible to the neuropathic effects of chlorpyrifos. The ratio AChE I_{50}/NTE I_{50} is predictive of OPIDP potential in vivo and allows extrapolation across species (Lotti and Johnson 1978). Even though this ratio cannot be precisely calculated for chlorpyrifos-oxon because of the presence of hydrolysing A-esterases in the nervous tissue, the relative potency of this compound in inhibiting AChE and NTE is very similar in both species. Also A-esterase activities seem similar in both hen and human nervous tissue homogenates.

In conclusion, chlorpyrifos causes OPIDP both in man and hen at doses well above the LD_{50}. Consequently, it is unlikely that OPIDP would develop in either species without a preceeding severe cholinergic syndrome. In vitro studies suggest that there are no major differences between man and hen as far as target enzyme sensitivity to chlorpyrifos-oxon. Because of the slow disposal of oral doses of chlorpyrifos, biochemical effects are delayed. Since this might also occur after dosing with other OPs, the predictive value of NTE measurement for OPIDP risk assessment might be hampered.

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