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Effects of Structure, Atropine, and Dosing Regimen on the Delayed Neurotoxicity of the Insecticide EPN

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EPN [EPN-ethyl; O-4-nitrophenyl O-ethyl phenylphosphonothionate] is a commercial organophosphorus ester [OP] insecticide causing neurological damage in humans (Xintaras and Burg 1980) and classical OP-induced delayed neuropathy [OPIDN] in hens (Abou-Donia and Graham 1979). The minimum effective neurotoxic dose of EPN is of concern because the heavy agricultural use of EPN on cotton provides considerable opportunity for occupational exposure. There is also interest in the relative neurotoxic potential of pairs of O-methyl and O-ethyl phenylphosphonothionates, since 5- to 10-fold differences in activity are known to occur, and their basis is obscure (Hansen et al 1985, Johnson et al 1986).

In hens, the acute oral LD_{50} of EPN is approximately 10 mg/kg, while the effective po dose for inducing irreversible overt ataxia is approximately 100 mg/kg (Abou-Donia and Graham 1979). EPN-ethyl is nevertheless a highly efficient neurotoxicant, causing paralysis in hens after 90 doses of 1.3 mg/kg/ day percutaneously or 90 doses of 3-4 mg/kg/day po (Francis et al 1982). EPN-ethyl was a more effective delayed neurotoxicant than leptophos [O-2,5-dichloro-4-bromophenyl O-methyl phenylphosphonothionate], and less neurotoxic, but more acutely toxic, than EPN-methyl [O-4-nitrophenyl O-methyl phenylphosphonothionate] (Francis et al 1982). However, hens in multiple dosing regimens in our laboratory were not concurrently treated with atropine, and Chrzanowski and Jelinek (1981) suggest that atropine may potentiate the delayed neurotoxicity of OPs by delaying their excretion. The increased persistence in the organism could increase the inhibition of NTE to critical levels, simulating the events in multiple dosing regimens (Johnson and Richardson 1984).

To establish the neurotoxic potential of EPN-ethyl and EPN-methyl relative to each other and to the human neurotoxicant leptophos, we determined the minimum po neurotoxic dose of EPN-ethyl and EPN-methyl, using hens comparable to those of our multiple dosing regimens. To assess the hypothesis that atropine alters the neurotoxic potential of OPs, we compared the onset of ataxia in hens exposed to desbromoleptophos [O-2,5-dichlorophenyl O-methyl

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phenyl phosphonothionate] with and without concurrent atropine administration. Unlike EPN-ethyl, desbromoleptophos causes OPIDN at doses 1-2 orders of magnitude below its LD_{50} (Francis et al 1982) and it is therefore possible to compare the effects of atropine on marginally neurotoxic doses.

MATERIALS AND METHODS

White leghorn hens were acquired at 20 weeks of age and held for 4-6 weeks until egg laying was well established. Layer mash, supplied by the University of Illinois Poultry Farms, and water were provided ad libitum at all times. Hens were treated with a single po dose of desbromoleptophos [O-2,5-dichlorophenyl O-methyl phenylphosphonothionate], EPN-ethyl [O-4-nitrophenyl O-ethyl phenylphosphonothionate] or EPN-methyl [O-4-nitrophenyl O-methyl phenylphosphonothionate]. The OPs were administered in gelatin capsules; no carrier was used. Atropine sulfate [Sigma Chemicals] was prepared in distilled water, 100 mg/ml, and was administered to hens ip immediately before treatment with the OPs, and symptomatically for up to 4 days after treatment with EPN-ethyl or EPN-methyl.

Hens were examined at least once each day, and their ability to walk characterized on a 5 point scale in which 0 = normal gait and behavior; +1 = suspicion of abnormal gait; +2 = overt ataxia and stumbling; +3 = severe ataxia with total inability to stand; +4 = paralysis (Francis et al 1980, 1982, 1985). Hens were kept for at least 30 days after treatment to determine the degree of recovery from ataxia.

RESULTS AND DISCUSSION

When desbromoleptophos [DBL] was administered to hens at doses of 20 to 30 mg/kg, minor disturbances of gait were seen for 24 hours after treatment. Transient stage +2 delayed ataxia was seen in 3 of 6, and permanent delayed ataxia [final status +2] occurred in 1 of 6, hens treated at 20 mg/kg. Neither of the hens treated at 30 mg/kg developed even transient stage +2 ataxia, and 4 of 4 hens treated with 50 mg/kg developed permanent ataxia [final status +2], progressing to paralysis [+4] in 2 of 4 hens. As can be seen in Table 1, the severity of ataxia within doses was not dependent on atropine administration. In earlier studies, the minimum effective neurotoxic dose of DBL was also between 20 and 30 mg/kg (Sanborn et al 1977, Metcalf et al 1983); the precise dose appears to vary with individual hens, rather than with atropine administration. At higher doses [50 or 100 mg/kg], neither the initial weakness on day 1-3 after treatment, nor the severity of the permanent ataxia, correlated with atropine treatment.

For both EPN-methyl and EPN-ethyl, permanent stage +2 ataxia occurred only with treatment approaching 200 mg/kg, despite profound and long-lasting debility seen at all levels of treatment [Tables 2, 3]. Since 1 hen treated with 200 mg/kg EPN-methyl and 1 hen treated with 200 mg/kg EPN-ethyl recovered to stage +1/+2, this seems to be the minimum effective neurotoxic dose for both

Table 1. Effects of desbromoleptophos given in a single oral dose with and without atropine.

Hen No.	Atropine[1] (mg/hen)	DBL[2] (mg/kg)	Final Status	Days to Reach Stage: +1	+2	+3	+4	Death
718	50	20	+1	9	17	--	--	--
733	--	20	+1/+2	11	15	--	--	--
734	--	20	+2	4	15	19	--	--
758	50	20	+1	10	--	--	--	--
731	--	20	+1	12	--	--	--	--
708	50	20	+1	3	--	--	--	--
752	50	30	0/+1	10	--	--	--	--
701	--	30	0/+1	1	--	--	--	--
727	50	50	+4	2	1	--	15	--
729	50	50	+3/+4	2	15	17	--	--
730	--	50	+3/+4	2	12	17	--	--
728	--	50	+4	1	10	12	17	--
726	--	100	+4	2	1	12	15	--
725	50	100	+3/+4	3	2	18	--	--

[1] Atropine was administered ip at the time of desbromoleptophos administration. No subsequent doses of atropine were administered because hens did not exhibit cholinergic symptoms.

[2] Desbromoleptophos was administered po, in a single dose, without carrier, in a gelatin capsule.

compounds in our hens. These data essentially confirm the estimation of Abou-Donia (1979) that the single po dose of EPN-ethyl that induces OPIDN lies between 100 and 200 mg/kg. The somewhat greater resistance of our hens to paralysis is almost certainly due to their younger age, since sensitivity to OPIDN is known to increase with age (Johnson and Barnes 1970).

The similarity of neurotoxic potential seen in the present acute dosing comparison of EPN-methyl and EPN-ethyl is not seen in multiple dosing regimens (Francis et al 1980, Francis et al 1982, Francis et al 1985). In those studies, EPN-methyl, administered at 10 mg/kg/day, caused permanent stage +2 ataxia in 1 of 2 hens; the other recovered to stage +1. At 5 mg/kg/day, EPN-methyl induced transient stage +2 ataxia after 33 days in 2 of 2 hens, but both recovered. EPN-ethyl, in contrast, induced permanent stage +2 ataxia in 4 of 5 hens treated with 5 mg/kg/day for 90 days; the 5th hen died after 30 doses. EPN-ethyl also induced permanent ataxia [stage +2] in 1 of 3 hens treated orally at 4 mg/kg/day for 90 days.

The contrast between EPN-ethyl and EPN-methyl in acute and multiple dosing regimens is pertinent both to the problem of regulating occupational exposure of delayed neurotoxicants, and to the

Table 2. Effect of EPN-ethyl given in a single oral dose with atropine to protect against cholinergic effects.

Hen No.	Atropine[1]: (mg/hen)	EPN[2] (mg/kg)	Final Status	Days to Reach Stage: +1	+2	+3	+4	Death
404	50	--	0/+1	8	6	--	1	--
406	100	--	0/+1	1	--	--	--	--
846	20	--	0	1	--	--	--	--
815	6	50	0/+1	26	22	19	1	--
816	6	60	0/+1	23	20	19	1	--
826	97	75	0/+1	17	16	--	1	--
848	100	75	--	--	--	--	--	1
863	105	75	0/+1	16	6	4	1	--
847	107	75	0/+1	15	5	--	1	--
405	100	100	0/+1	7	6	--	1	--
407	130	100	+1	8	7	--	1	--
722	50	100	+1	10	6	--	1	--
898	50	100	+1	6	--	4	1	--
723	100	110	0/+1	8	7	6	1	--
408	100	150	+1/+2	9	8	7	1	--
732	450	200	+2	--	12	11	1	--
736	200	200	+1	11	10	--	1	--
409	300	200	+3/+4	--	12	11	1	--
410	50	200	--	--	--	--	--	1

[1] Atropine was administered ip at the time of EPN-ethyl administration and thereafter when hens exhibited cholinergic symptoms. Cumulative dose is shown.

[2] EPN-ethyl was administered in a single po dose, in a gelatin capsule without carrier.

process of risk assessment. If only the acute dosing regimen is considered, EPN-ethyl and EPN-methyl are similar to each other and to leptophos, since the latter has a minimum effective neurotoxic dose of approximately 250 mg/kg in our hens (Sanborn et al 1977, Francis et al 1982). In multiple dosing regimens, EPN-methyl is similar to leptophos in that both induce permanent ataxia [stage +2] when given at 10 mg/kg/day, but not at 5 mg/kg/ day, for 90 days. Both diverge from EPN-ethyl, which is seen to be considerably more neurotoxic in multiple dosing regimens.

Therefore, the data argue strongly for the use of multiple as well as acute dosing regimens to evaluate neurotoxic potential of OPs. This is particularly pertinent for the relatively persistent OPs like EPN, isofenphos and chlorpyrifos, which have been shown to be delayed neurotoxicants at levels above their LD_{50} in hens (Hixson 1984, Wilson et al 1984), and are persistent in the

Table 3. Effect of EPN-methyl given in a single oral dose with atropine to protect against cholinergic effects.

Hen No.	Atropine[1]: (mg/hen)	EPN[2] (mg/kg)	Final Status	Days to Reach Stage: +1	+2	+3	+4	Death
846	20	--	0	1	--	--	--	--
404	50	--	0/+1	8	6	--	1	--
406	100	--	0/+1	1	--	--	--	--
813	4	50	0/+1	3	2	--	--	--
814	4	50	0/+1	2	1	0	--	--
724	100	50	0/+1	8	7	5	1	--
844	18	75	+1	4	3	1	--	--
849	14	75	0	5	3	2	1	--
415	100	100	0/+1	9	6	--	1	--
770	350	180	+1/+2	11	--	10	1	--
412	300	200	+1/+2	--	12	10	1	--
411	300	200	+4	--	--	8	1	--
739	400	200	+4	--	13	10	1	--

[1] Atropine was administered ip at the time of EPN-methyl administration and thereafter when hens exhibited cholinergic symptoms. Cumulative dose is shown.

[2] EPN-methyl was administered po, in a single dose, without carrier, in a gelatin capsule.

organism, but for which few data on multiple dosing regimens are available (Francis et al 1985). For such compounds, regulatory reliance on the high single doses needed to cause neuropathy may be disastrously optimistic.

Not only inhibition of NTE, but also aging of the OP-NTE complex is thought to be required for OPIDN to develop (Johnson and Richardson 1984). Both differential absorption from the GI tract and differential aging between O-methyl and O-ethyl analogs of leptophos have been suggested as the basis for the significantly greater *in vivo* neurotoxicity of leptophos-methyl compared to leptophos-ethyl (Hansen et al 1985, Johnson et al 1986). The data from the present study, of hens treated with the O-methyl and O-ethyl analogs of EPN, suggest that differences between O-ethyl and O-methyl phosphonates must be evaluated separately for each analogous pair, since --- in contrast to the 5-fold difference between leptophos-ethyl and leptophos-methyl --- there is essentially no difference between single dose of EPN-methyl and EPN-ethyl that induces neuropathy.

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