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11. The latter classification was determined by requiring each subject to write a sentence on lined paper that was aligned vertically with the edge of the table. If the pen pointed toward the subject and the hand was held above the line of writing, the subject was classified as an inverter. If the pen pointed away and the hand was held below the line of writing, the subject was classified as a noninverter.
12. This was, a simple reaction-time procedure in which the subject responded whenever a stimulus was present and withheld a response on catch trials. Because stimulus discrimination was not involved, the procedure was considered a Donders type a.
13. Each session was divided into four blocks of an equal number of trials. Equal numbers of stimulus and blank trials were in each block and they varied randomly with each other. The order of presentation followed a predetermined random sequence. Experimental trials were preceded by 25 practice trials. Short breaks were permitted between blocks or whenever the subject complained of fatigue or boredom. Ideally the experiment required two 1.5-hour sessions per subject with two modalities tested per session, but deviations occurred because of scheduling problems.
14. Two solenoids were attached by sliding collars to two vertical posts mounted on separate 12.7 by 12.7 cm Plexiglas bases. A Grason-Stadler power supply (Model E1100DA) was used, and the onset and duration of solenoid presentation was controlled by a Gerbrands series 300 integrated-circuit millisecond timer.
15. To provide a relatively fixed interval between warning signal and trial in the visual and tactual conditions, a centrally placed Birkbeck timer and signal source repeated 50-msec bursts of noise at 1-second intervals. The experimenter gave the warning signal simultaneously with one burst and presented the stimulus on the succeeding burst. The subject was made aware of this procedure.
16. The sinusoidal tones were computer-generated and aligned and played at an intensity of 86 dB (sound level) measured by a General Radio 1565-A sound level meter through a 1560-P82 earphone coupler.
17. The means and standard deviations were calculated for each session for each subject. Because we wished to discard those trials on which the subject was either inattentive or anticipated stimulus presentation, we excluded all trials in which the reaction times were 2 standard deviations different from the mean. The mean was then recalculated. Typically, about 1.5 trials in 50 were excluded. This procedure affected the outcome, in terms of which sensory field was favored for a given response hand, in only one subject. Also, if standard deviations were very high (more than 100 msec) or more than ten errors were committed during one session, we assumed that the subject was inattentive and the session was rerun. This was necessary for only four of the 222 sessions.
18. Separate analyses of variance were made for each modality, with group as the between-subject variable and responding hand and sensory field as the within-subject variables. The single inverted right-hander could not be included in the analysis.
19. Neither responding hand nor field of stimulation appears to have clearly influenced reaction times, except in the tactual modality where reaction times tend to be shorter for right field stimulation [$F(1, 33) = 6.49, P = .16$]. The latter finding is irrelevant with regard to the hypotheses under consideration.
20. The latency differences between sensory fields were small and close to electrophysiological measures of interhemispheric transmission times [F. Bremer, *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* **36**, 424 (1958); B. Grafstein, *J. Neurophysiol.* **22**, 504 (1959); L. T. Rutledge and T. T. Kennedy, *Exp. Neurol.* **4**, 470 (1961); H. Teitelbaum, S. W. Sharpless, R. Byck, *J. Comp. Physiol. Psychol.* **66**, 623 (1968); A. Salamy, *Science* **200**, 1409 (1978) and references therein; A. Ledlow, J. M. Swanson, M. Kinsbourne, *J. Exp. Psychol. Hum. Percept Perform.*, in press]. Consequently they are likely to reflect neuroanatomical relations rather than differences in spatial compatiblity between stimulus and response where latency differences, as well as overall response times, are much longer [D. E. Broadbent and M. Gregory, *J. Exp. Psychol.* **63**, 575 (1962); R. J. Wallace, *ibid.* **88**, 354 (1971); J. Callan, D. Klisz, O. A. Parsons, *ibid.* **102**, 1039 (1974)]. Lastly, the simple reaction-time procedure we employed corresponds to a Donders type a which is not prone to significant spatial compatibility effects [G. P. Anzola, G. Bertolini, H. A. Buchtel, G. Rizzolatti, *Neuropsychologia* **15**, 295 (1977); G. Berlucchi, F. Crea, M. DiStefano, G. Tassinari, *J. Exp. Psychol. Hum. Percept. Perform.* **3**, 505 (1977)].
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24. One possibility is that the visual projections are uncrossed. Although such anomalies have been found in albinos [D. Creel, F. E. O'Donnell, Jr., C. J. Witkop, Jr., *Science* **201**, 931 (1978)], preliminary electrophysiological evidence makes this possibility very unlikely in our population. A more likely alternative is that the anomaly lies at the interface of the visual and motor systems. In this connection, W. Richards' study [*Exp. Brain Res.* **17**, 333 (1973)] on subcortically mediated stereoscopic depth perception in individuals with scotomata of occipital origin may be instructive. In one individual, the identical stimulus lateralized to a scotomatous half-field changed from being reported in front to in back of the plane of fixation only when he responded with the hand opposite the stimulated field. This suggested to Richards that there may be contralateral inhibitory somatosensory-visual connections in the tectum that influence perception in the contralateral field. One has simply to assume ipsilateral influences in inverted left-handers to account for the results of our experiments as well as the observation that inverted writing occurs most in cultures where the direction of writing is from left to right. However, this hypothesis is speculative and requires much more research to substantiate.
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Delayed Neurotoxicity of Phenylphosphonothioate Esters

Abstract. *Administration of a single oral dose of five phenylphosphonothioate esters produced delayed neurotoxicity in hens; their potency was, in descending order, cyanofenphos, EPN, desbromoleptophos, leptophos, and EPBP (Seven). Histological examination showed that in some hens there was marked axonal and myelin degeneration in the spinal cord and peripheral nerves. The results suggest that delayed neurotoxicity may be a general feature of phenylphosphonothioate insecticides.*

Although organophosphorus pesticide-induced delayed neurotoxicity may be produced by some organophosphorus esters, most of these compounds cause acute poisoning with temporary muscle weakness (*1*). The delayed neurotoxic effect was first demonstrated in humans (*2*); later, some additional species were found to be susceptible (cats, dogs, cows, and chickens), while others were not (rodents and some primates) (*3*). The test animal chosen to demonstrate this syndrome is the adult chicken. The clinical condition is recognized as ataxia, which develops 4 days or more after administration. Lesions are characterized by degeneration of axons with subsequent Wallerian degeneration of myelin. Recently, the phenylphosphonothioate insecticide leptophos has been implicated in the poisoning and paralysis of some workers in Texas (*4*). Leptophos produces delayed neurotoxicity in farm animals and chickens (*5–9*). A photodegradation product of this compound, desbromoleptophos (DBL), was reported to cause delayed neurotoxicity in chickens (*10*). Another insecticide in this group, EPN, caused delayed neurotoxicity when fed (*11*) or orally administered (*12, 13*) to chickens. I report here the ability of two other phenylphosphonothioate insecticides, EPBP and cyanofenphos, to cause delayed neurotoxicity in hens. I also report the relative neurotoxic potencies of the five phenylphosphonothioate esters: leptophos, DBL, EPBP (*S*-Seven), EPN, and cyanofenphos (Fig. 1).

Experiments were performed with adult hens (*Gallus gallus* domesticus), mixed breed (Spafas, Inc., Norwich, Connecticut), each 1 year old and weighing approximately 1.5 kg. Eight groups of hens (three each) were given a single oral dose of each compound in a gelatin capsule; dose ranges (in milligrams per kilogram of body weight) were: leptophos, 10 to 3000; DBL, 10 to 100; EPBP, 100 to 5000; EPN, 10 to 500; and cyanofenphos, 5 to 250. Hens given all doses of EPN and high doses of the other insecticides had been previously treated with a single oral dose of atropine sulfate (30 mg/kg) in water as protection against the acute toxicity of these esters. Controls consisted of four groups of hens orally given empty gelatin capsules, tri-*o*-cresyl phosphate (TOCP) (500 mg/kg), parathion (10 mg/kg) with atropine sulfate (30 mg/kg), or atropine sulfate (300 mg/kg). The birds were supplied with food and water ad libitum. Body weights were monitored and hens were examined periodically for neurological signs of delayed neurotoxicity. Nerve tissues were

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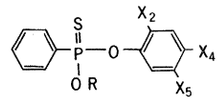
excised and prepared for histological examination in birds that died during the experiment and in birds killed by heart puncture at the end of the 60-day test period (*6*). Sections from brain, spinal cord, and sciatic nerve (8 μm) were stained with hematoxylin and eosin combined with luxol fast blue. Sections from the peripheral nerves were also stained with Holme's silver stain.

The results show that EPN was the most acutely toxic to hens, followed in diminishing order by cyanofenphos, DBL, leptophos, and EPBP. All hens that survived these insecticides developed ataxia within 4 to 21 days after administration. Some of the hens that were given high doses of EPN had previously recovered from an initial paralysis, which presumably resulted from inhibition of acetylcholinesterase. After the ataxia, the clinical condition of hens given high doses progressed to paralysis and, in some hens, death. All TOCP-treated hens developed ataxia and their condition progressed to paralysis. In contrast, parathion-treated hens showed initial leg weakness, but recovered within a few days. All controls that received atropine sulfate or gelatin capsules remained normal. The result that EPN, the most acutely toxic of the tested esters [dose lethal to 50 percent of a population (LD_{50}) = 10 mg/kg (*12*)], was 2.5 times less potent than cyanofenphos [LD_{50} = 540 mg/kg (*14*)] in producing delayed neurotoxicity in hens (Table 1) is in harmony with the conclusion that acetylcholinesterase is not directly involved in the etiology of delayed neurotoxicity (*15*). The potency of the other esters in causing a delayed neurotoxic effect, however, generally paralleled their potency in causing acute effects. These results are in accord with the hypothesis that the initial event in delayed neurotoxicity induced by an organophosphorus compound may be a phosphorylation of a nucleophilic site on the protein target (*8, 16*).



Compound	R	X_2	X_4	X_5
Leptophos	CH_3	Cl	Br	Cl
DBL	CH_3	Cl	H	Cl
EPBP	C_2H_5	Cl	Cl	H
EPN	C_2H_5	H	NO_2	H
Cyanofenphos	C_2H_5	H	CN	H

Fig. 1. Structural formulas of leptophos, DBL, EPBP, EPN, and cyanofenphos.

The clinical condition and histological changes found in this study are identical to those reported for other compounds producing delayed neurotoxic effects, such as TOCP (*1*). Axonal and myelin degeneration were readily seen in tibial and peroneal nerves below the division of the sciatic nerve into separate nerves. Degeneration of the anterior columns of thoracic and lumbar spinal cord was the most consistent histological correlate of delayed neurotoxicity. This study confirms earlier results (*6–9, 17*) showing that the lesions in the peripheral nerve occur earlier than those in the spinal cord as seen by the light microscope. This might be attributed in part to the fact that the peripheral axons can regenerate, whereas those in the central nervous system cannot. The observation that the spinal cord lesions were not seen until later suggests that this effect is even more delayed than the degeneration of the peripheral nerves. In humans, the symptoms of such spinal cord injury would resemble those of multiple sclerosis, and most of the factory workers exposed to leptophos were initially diagnosed as having multiple sclerosis (*4*). The extent of injury and progression of symptoms depend on the dose and duration of exposure. The clinical condition may improve as the result of peripheral nerve regeneration or restoration of function of the spinal cord neurons; that is, other cells having the same function may be adequate to maintain normal activity, or other neurons may acquire the needed function. When neither course is possible—for example, in extensive damage of the spinal cord—some loss of function may result.

Aromatic organophosphorus esters that induce delayed neurotoxicity at large doses apparently undergo metabolic activation to more potent neurotoxic metabolites. This hypothesis is supported by the finding that TOCP, which requires a single oral dose of 500 mg/kg to cause delayed neurotoxicity, is metabolized in vivo to the active neurotoxic agent *o*-cresyl saligenin phosphate (*1*). This metabolite causes delayed neurotoxicity at a dose of 1 mg/kg (*18*). The hypothesis is also in accord with the result that DBL, which is a photodegradation product of leptophos (*10*), is twice as effective a delayed neurotoxic agent as leptophos in chickens.

It has not been possible to predict, on the basis of chemical structure, whether a compound will cause delayed neurotoxicity. Although most of these compounds are cholinesterase inhibitors, inhibition of this enzyme does not seem to be related to delayed neurotoxicity. The results reported here strongly support the suggestion (*19*) that delayed neurotoxicity is characteristic of phenylphosphonothioate esters. This should be taken into consideration in designing, preparing, handling, and registering new organophosphorus esters.

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Table 1. Delayed neurotoxic effect, relative neurotoxicity, and LD_{50} values of phenylphosphonothioate esters. Administration of a single oral dose was followed by a 60-day observation period. Chemicals used were technical grade (infrared, 94.8 percent; gas chromatography, 87.2 percent) *O*-methyl *O*-4-bromo-2,5-dichlorophenyl phenylphosphonothioate (leptophos), pure *O*-methyl *O*-2,5-dichlorophenyl phenylphosphonothioate (desbromoleptophos, DBL), technical grade (96.43 percent) *O*-ethyl *O*-2,4-dichlorophenyl phenylphosphonothioate (*S*-Seven, EPBP), pure *O*-ethyl *O*-4-cyanophenyl phenylphosphonothioate (Surecide, cyanofenphos), technical grade (85 percent) *O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate (EPN), tri-*o*-cresyl phosphate (TOCP), and *O*,*O*-diethyl *O*-4-nitrophenyl phosphorothioate (parathion).

Compound	Dose range (mg/kg)	Threshold dose* (mg/kg)	Relative neurotoxicity	LD_{50} (mg/kg)	Reference
Cyanofenphos	5–250	10	80	540	(*14*)
EPN	10–500	25	32	10	(*12*)
Desbromoleptophos	10–100	50	16	N.A.†	
Leptophos	10–3000	100	8	4700	(*5*)
EPBP	100–5000	800	1	>5000‡	

*The minimum single oral dose that caused clinical signs of delayed neurotoxicity. †N.A., data are not available. ‡M. B. Abou-Donia, unpublished data.

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Inosine May Be an Endogenous Ligand for Benzodiazepine Receptors on Cultured Spinal Neurons

Abstract. *Mouse spinal neurons grown in tissue culture were used to study the membrane effects of the benzodiazepine flurazepam and the naturally occurring purine nucleoside inosine, which competes for benzodiazepine receptor sites in the central nervous system. Application of inosine elicited two types of transmitter-like membrane effects: a rapidly desensitizing excitatory response and a nondesensitizing inhibitory response. Flurazepam produced a similar excitatory response which showed cross-desensitization with the purine excitation. Flurazepam also blocked the inhibitory inosine response. The results provide electrophysiological evidence that an endogenous purine can activate two different conductances on spinal neurons and that flurazepam can activate one of the conductances and antagonize the other.*

Benzodiazepines are a commonly used class of drugs with a broad spectrum of application as tranquilizers, anticonvulsants, muscle relaxants, and sedatives (*1*). These therapeutic effects are presumably mediated in part through actions on the central nervous system (CNS) since high-affinity, stereospecific, and saturable binding sites for benzodiazepines have been identified throughout the CNS both in vitro (*2*, *3*) and in vivo (*4*). Several substances isolated from the CNS compete with tritiated benzodiazepines for CNS receptor sites (*5*). These endogenous substances have been identified as the purine hypoxanthine and its nucleoside inosine (*6*), both of which are synthesized de novo in neuronal tissue (*7*). In addition to their function in nucleic acid synthesis, purines can be released from nerve tissue (*8*, *9*) and alter the excitability of central (*10*) and peripheral neurons and smooth muscle (*11*), results suggesting that these compounds may mediate intercellular communication in the nervous system (*9*–*11*). We have used cultured spinal neurons to examine the membrane actions of the benzodiazepine flurazepam and the purine inosine and report that (i) these substances produce rapidly desensitizing excitatory responses that cross-desensitize with each other, and (ii) inosine inhibits excitability by increasing membrane conductance, an effect that is blocked by flurazepam. The results provide evidence for two transmitter-like effects of inosine and suggest that flurazepam can act as an agonist at one site and antagonist at the other.

Neurons were dissociated from spinal cords of 13-day-old fetal mice and grown in tissue culture (*12*, *13*). Intracellular recordings were made on the modified

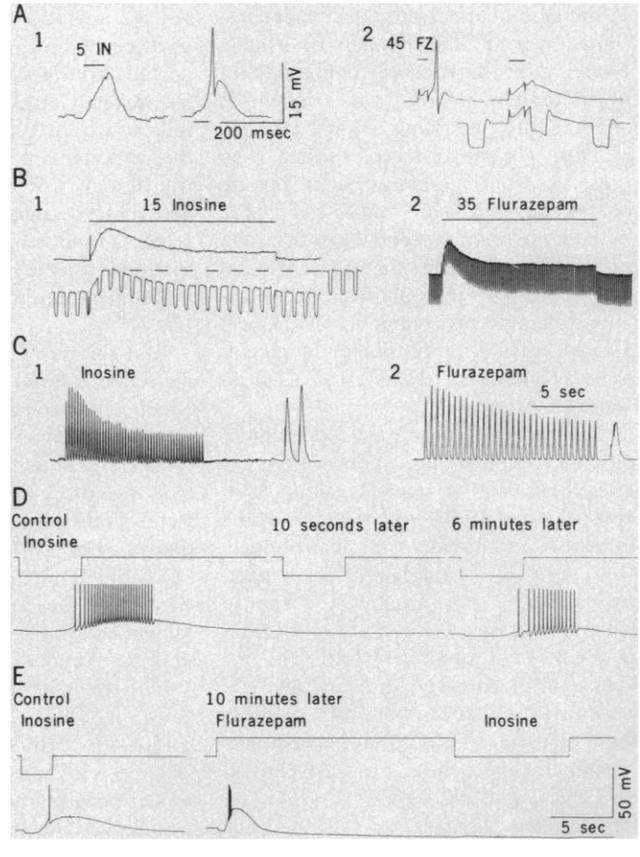

Fig. 1. Comparison of excitatory responses to inosine (*IN*) and flurazepam (*FZ*) on cultured spinal neurons. Pen recorder traces of potassium acetate recordings from eight cells are shown. Bars above traces in (A to C) and Fig. 2 indicate iontophoretic drug application periods, with numbers above bars giving iontophoretic currents in nanoamperes. Voltage calibration bar in (A1) refers to (A) to (C) and time calibration bar in (C2) refers to (B) and (C). (A1) Depolarizing responses to inosine at −62 mV (left) and −55 mV (right) are shown, the latter sufficient to generate a spike whose amplitude is attenuated by the frequency response of the pen recorder. (A2) Flurazepam responses recorded on another cell show excitation at −48 mV (left) and little change in conductance at −55 mV (as reflected in the amplitude of the voltage response to −0.5-nA hyperpolarizing pulses). (B) In sustained applications of inosine (B1) and flurazepam (B2) to two different cells, desensitization of both voltage and conductance changes is shown, the latter assessed with constant −0.4-nA (B1) and −0.5-nA (B2) pulses. Membrane conductance at depolarized level is less than that during inosine application (inset in B1). (C) Repetitive applications of inosine (C1) at 175 nA in 8-msec pulses (four per second) and of FZ (C2) at 150 nA in 40-msec pulses (two per second) on two cells show initial potentiation of response amplitude followed by desensitization during both trains. Complete recovery within 10 seconds is shown in (C1). [Recording speed for last two responses in (C1) and final response in (C2) was five times faster.] (D) Application of inosine by pressure (monitored above voltage trace) from a pipette containing 10 μM inosine causes a brief excitation which completely desensitizes membrane to an inosine application 10 seconds later. Partial recovery is evident 6 minutes later. (E) Cross-desensitization between FZ and inosine is shown. Pressure-applied inosine briefly excites a spinal cord neuron (*Control*); 10 minutes later sustained pressure application of FZ from a 10-μM pipette causes an excitatory response which rapidly desensitizes. The excitatory response to inosine is then completely abolished. The inosine response recovers 10 minutes after termination of FZ application. Membrane potentials (in millivolts): (B1), −60; (B2), −62; (C1), −56; (C2), −60; and (D) and (E), −61.

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