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COLLEGE OF AGRICULTURAL AND
ENVIRONMENTAL SCIENCES
AGRICULTURAL EXPERIMENT STATION
DEPARTMENT OF AVIAN SCIENCES

DAVIS, CALIFORNIA 95616

January 18, 1983

Dr. Larry Chitlik
US-EPA, HED, Toxicology Branch
Cm-2, Room 820-B
401 M St SW
Washington, D.C.
20460

Dear Dr. Chitlik:

Enclosed is a copy of our interim report to CDFA concerning isofenphos, antidotes and delayed neurotoxicity, and a copy of a letter of request for information from D.J. Clegg, Head, Pesticides Section, Toxicological Evaluation Section, Canadian Bureau of Chemical Safety.

I hope you find the report useful. I am taking the liberty of directing to you all inquiries from EPA staff concerning the report itself. Please let me know if you or your colleagues have any questions concerning the work.

Sincerely yours,

Barry W. Wilson
Professor

cc: L. Lewis, Dir. UC Exp. Sta.
J. Knaak, CDFA
L. Johnston, CDFA

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DAVIS, CALIFORNIA 95616

January 18, 1983

Dr. D.J. Clegg
Head, Pesticides Section
Toxicological Evaluation Division
Bureau of Chemical Safety
Health and Welfare Canada
Ottawa, Ontario, K1A 0L2

Dear Dr. Clegg:

Thank you for your inquiry of January 5, 1984, concerning our research on isofenphos (IFP). We began studying the agent this fall at the request of California Department of Food and Agriculture (CDFA) after questions were raised concerning the efficacy of licensed antidotes for organophosphorus esters with IFP. During our studies of the antidotes 2-PAM and atropine we discovered that levels of IFP of 100 mg/kg and above given subcutaneously were neuropathic to two strains of laying hens. We notified state officials as soon as we suspected the problem; they, in turn, notified Mobay. After several of Mobay's staff and state officials visited my laboratory, CDFA Assistant Director Magee issued a statement (and I prepared a press release) in November describing our preliminary findings and the state put a hold on the use of the agent in their Japanese Beetle eradication program. (A copy of my press release is enclosed.) By the end of December we had amassed enough data on the occurrence of Organophosphate Induced Delayed Neuropathy in hens treated with isofenphos, including inhibition of hen brain neurotoxic esterase, to present the results in our interim report on the project. A copy of this report is enclosed; I would be happy to answer any questions you might have concerning it.

Although at first CDFA expressed an interest in our doing 90 day repeated exposure studies of the agent, they subsequently decided against it, preferring to wait for the results of other studies. (It is my understanding that EPA has asked Mobay to run some new tests.) We are continuing to complete the antidote studies planned for in our contract with the state. Other research will be undertaken if funding permits.

We are in the final stages of finishing a manuscript to be submitted to the Bulletin of Environmental Contamination and Toxicology and would prefer that our particular findings not be publicized until the paper is in press. However, my colleagues and I understand the importance of our results to regulatory decisions and have freely discussed the conclusions of our research in public with others.

Sincerely yours,

Barry W. Wilson
Professor

cc: L. Lewis, Dir. UC Exp. Sta.
J. Knaak, CDFA
L. Johnston, CDFA
[redacted]

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DAVIS, CALIFORNIA 95616

December 28, 1983

Rex Magee
Assistant Director
Division of Plant Industry
Department of Food and Agriculture
1220 N Street, Sacramento CA 95814

Dear Director Magee;

Attached is the December Interim Report on the antidote project. Photomicrographs of the histology will be sent on when finished.

Please call me if you or your staff have any questions concerning the findings.

Sincerely,

Barry W. Wilson
Professor

bww/

INTERIM REPORT
DECEMBER 1983
TITLE: Antidotes and Isofenphos
AUTHOR: B.W. Wilson

INTRODUCTION AND SUMMARY

Isofenphos (IFP, Amaze, Oftanol, O-ethyl- O-2-isopropoxy-carbonylphenyl isopropylphosphoramidothioate), the organophosphate (OP) in Oftanol that is registered by the EPA for home and garden use, was applied in the fall of 1983 to soil in a suburb of Sacramento, California during a campaign to eradicate the Japanese Beetle. Questions as to the efficacy of the approved antidotes to OP poisoning, atropine and 2-pralidoxime (2-PAM), in treating acute exposure to IFP led to an antidote project on chickens, rats and guinea pigs exposed to the OP. This interim report presents the results of the first set of experiments. We found that the LD50 for IFP was approximately 5 mg/kg in the chicken and 45 mg/kg in the rat under the conditions of our experiments. Therapeutic injections of atropine and 2-PAM protected chickens to at least 15 times and rats to at least 2 times the LD50 levels of IFP. When prophylactic doses of the antidotes were used to protect hens to 20 and 30 times (100-150 mg/kg) the LD50 of IFP, clear cut behavioral, biochemical and morphological signs of organophosphate induced delayed neuropathy (OPIDN) appeared in most of the hens tested. The final stage of the project will involve studies of the effectiveness of the antidotes on guinea pigs and examination of the cholinesterase levels in blood, muscle and brain of IFP treated animals with and without antidotes.

METHODS

IFP (technical grade) was provided to CDFA by Mobay Inc, Kansas City, Kansas and administered in polyethylene glycol 400 (Sigma). Atropine sulphate (Sigma) and 2-PAM chloride (Aldrich) were prepared in distilled water.

IFP was studied in hens in two ways; laying White Leghorn hens (Hyline, 1 1/2 years old) were given the OP by gavage and then treated therapeutically with the antidotes. Pedigreed laying New Hampshire x White Leghorn crosses 5-6 months old developed by Dr. H. Abplanalp of the Department of Avian Sciences were first treated prophylactically with atropine, given IFP by the subcutaneous route, and then treated several times over a period of days with atropine and 2-PAM. All birds were raised and cared for in the Department of Avian Sciences according to National Research Council standards. Male Sprague-Dawley rats averaging 220 to 260 grams were obtained from Simmonsen, Gilroy, California, kept and cared for by the campus Animal Resource Service. IFP was administered by gavage ; atropine and 2-PAM were administered either i.m. or s.c. Food was withdrawn the night before animals were dosed by gavage.

Brains of IFP-treated chickens were examined for inhibition of "neurotoxic esterase" by a colorimetric assay (Ishikawa et al, 1983). Histopathological examination of nerves, spinal cord, brains and muscles of selected hens were performed after fixation by perfusion with paraformaldehyde and glutaraldehyde by Dr. R.J. Higgins, D.V.M.

Protocols for animal care and sacrifice were submitted to and approved by the Campus Veterinarian.

Animals were staged as to their locomotion by the following scoring: Stage 1, slight impairment; Stage 2, bird wobbles, trips, slips when walking or running; Stage 3, bird rests on its tibio-tarsi; some scoot backwards instead of walking; Stage 4, bird's legs are paralyzed, extended forward or sideways.

Polaroid photographs were obtained of many of the paralyzed birds.

Specialized terminology used in this report includes the following: LD50: the dose of IFP that kills half of the animals tested within several days; dosage: given as mg per kg of body weight; gavage: oral administration of a chemical through a tube; s.c.: subcutaneous, administration beneath the skin; i.m.: intramuscular, administration by injection into a large muscle; therapeutic: administration of antidotes after IFP; prophylactic: administration of antidotes both before and after administration of IFP.

RESULTS

Table 1 lists the chronology of the experiments to date on the project. Approximately two dozen separate experiments have been performed since the start of the project.

Acute Symptoms: Gavage Experiments on Hens and Rats

The experiments and disposition of hens used in this part of the study are shown in Table 2; they encompass LD50, antidote, and OPIDN studies on behavior, biochemistry and histology.

Mortality of chickens to graded doses of IFP is shown in Table 3. The LD50 level was between 3 and 5 mg/kg. Similar experiments showed an LD50 of approximately 40 -45 mg/kg for male rats (Table 4). Symptoms were those expected of an organophosphorus ester; tremors, weakness, gasping; special features were extensive salivation in the chicken and piloerection in the rat. Death did not always occur rapidly; some rats died up to 4 days after treatment

Examples of the antidote dosing schedules used in the chicken and rat experiments are listed in Table 5; the actions of the antidotes are shown in Table 6 for hens and Table 7 for rats. In both animals, repeated doses of atropine and 2-PAM raised the levels of the lethal doses of IFP. For example, in two experiments in which IFP was given by gavage to White Leghorn hens 4/5 hens survived 75 mg/kg dose of IFP (15 times the LD50). Only 2/5 hens survived at 100 mg/kg. Leg weakness and lethargy were two common symptoms of hens dosed with IFP. Some birds did not recover movement of their hind limbs for several days. A few never completely recovered; one bird treated with 100 mg/kg died after 11 days. Rats also responded to the antidotes. Treatment with atropine and 2-PAM for two and a half days following IFP raised the LD50 from 45 mg/kg to above 80 mg/kg (Table 7). We suspect that the antidotes would be even more effective if given for another day or two since much of the mortality occurred after the antidote injections ceased.

Acute Symptoms: Subcutaneous Injections

Subcutaneous injection of IFP coupled with prophylactic administration of atropine considerably reduced the toxicity of IFP to hens, enabling them to readily survive a dose of 100 mg/kg. These birds and the sole White Leghorn survivor of the 100 mg/kg gavage experiment were the first to demonstrate signs of OPIDN. Also, two of the five White Leghorn hens treated with 75 mg/kg lost the acute symptoms only to become slightly ataxic later, showing symptoms equivalent to Stage 2 of OPIDN.

OPIDN

Pronounced symptoms of OPIDN were obtained when hybrid chickens were

treated prophylactically with the antidotes and injected with IFP at 100 mg/kg (Table 8). Injection of 20 mg/kg atropine 30 minutes before and 50 mg/kg 2-PAM simultaneous with s.c. injection of IFP followed by periodic injections of the antidotes resulted in the survival of all (14/14) hybrid hens at 100 mg/kg. At first the hens were unable to stand; most regained use of their limbs within several days. Then, starting on days 10-14 many of the hens that had regained their mobility showed progressive symptoms of leg ataxia and paralysis. In an early stage of the disorder, birds were able to stand in the cages, but sank down onto their hocks (tibio-tarsi) when placed on a flat surface. Often the animals were unable to walk forward, scooting backward whenever they tried to move (Stage 3). Within a few days the birds became unable to stand in their cages, and later, the legs of some birds became permanently extended forward or sideways (Stage 4). Wings remained functional; the animals were alert, and ate and drank water when the cages were modified to make them available. Antidote and PEG injected hybrid birds exhibited mild behavioral signs of leg weakness; they stood and moved normally in the cages, but sat and moved little when placed on the floor.

Symptoms of OPIDN also were obtained with the White Leghorn hens. However, none of those treated at 100 mg/kg have progressed beyond Stage 3. An experiment examining the response of the White Leghorn hens to 100 and 150 mg/kg IFP after prophylactic dosing with atropine and 2-PAM is in progress. Two of three birds at 100 mg/kg and three of three birds at 150 mg/kg IFP survived past the first week. At 15 days, two of the birds at 150 mg/kg and one bird at 100 mg/kg were noticeably ataxic (Stage 3). Antidote injected controls exhibited no signs of leg weakness.

Strong evidence that IFP is neuropathic was obtained when the brains of treated animals were examined for NTE activity. Inhibitions as great as 85 % were found in the brains of hybrid birds exposed 3 days before to 100 mg/kg of IFP (Table 9). The NTE of hens injected with antidote and PEG was not affected. Similar findings were made with the White Leghorn hens, but the number of them available for such a study was too few for a complete experiment.

Histological examination of nerves of the leg, spinal cord and brain of hybrid chickens confirmed the presence of nerve damage in treated birds. Lesions characteristic of OPIDN were found in the distal regions of the sciatic nerves (i.e.axonal swelling, segmented spheroid vacuolization, degeneration and phagocytosis of myelin sheaths), spinal cord (i.e. bilateral lesions in the gracilis tract of the dorsal columns) and brain (i.e. spinocerebellar tracts of medulla oblongota) of four birds exposed 3-4 weeks before to 100 mg/kg of IFP and showing Stage 4 symptoms of leg paralysis. The incidence and severity of the lesions in the peripheral nervous system increased the further the axon was from the spinal cord; for example, there was approximately a 1 % incidence of lesions in the ischiatic nerve near to the spinal cord, and approximately 40% incidence of lesions in the lateral metatarsal nerve near the toes. The positive controls, birds treated with TOCP showed similar lesions; the antidote injected and untreated controls exhibited no significant microscopic lesions. No lesions were found in the nerves of one hen injected with antidote and PEG and one uninjected control bird. The photomicrographs of the histology were not fnished in time for this report and will be sent on as an addendum later.

Damage to the lateral adductor muscle was seen in some of the OP treated birds, attributable to the cutting off of circulation (ischemia) to the muscle caused by the squatting position of the paralyzed birds.

DISCUSSION

The results indicate that atropine and 2-PAM are useable antidotes to IFP poisoning in rats and excellent antidotes to IFP poisoning in hens. Atropine and 2-PAM were effective in protecting rats from IFP though not to as great an extent as it protected the chickens. One possibility is that the IFP has almost totally inhibited tissue AChE activity and that antidote should be given to the animals for the several days it takes for the rat to resynthesize AChE. These findings differ from the conclusions presented in the FAO Summary Report of confidential data on IFP (1982). The report said that neither atropine, 2-PAM or toxigonin "was effective as an antidote..." in situations where atropine sulphate and 2-PAM were both given at 50 mg/kg, 45 to 120 minutes after single oral toxic doses of IFP.

We also found that the LD50 of IFP was between 3 and 5 mg/kg in hens, a quarter that reported in the FAO report, and that repeated doses of atropine and 2-PAM protected most of the hens tested against doses as high as 75 mg/kg therapeutically (4/5 survived) and 150 mg/kg (3/4 survived) prophylactically. The FAO report reported that the oral LD50 for IFP in the chicken was 21 mg/kg and that prophylactic administration of atropine alone elevated it to 74 mg/kg (in other words, approximately half the IFP treated birds died at 75 mg/kg after treatment with atropine).

One probable reason for the differences in our results with antidotes and the FAO Report is that we gave the animals repeated doses of the antidotes exending over a period of several days.

Another difference between our conclusions and those in the FAO Report concerns long-term neural damage. The report concluded that IFP was not neuropathic based on their finding that atropine-treated hens surviving 75 mg/kg did not develop OPIDN. The greater effectiveness of atropine plus 2-PAM over atropine alone as an antidote to acute poisoning by IFP enabled us to treat hens at s.c. high doses resulting in intense neuropathic symptoms developing in animals given 100-150 mg/kg of IFP, 20-30 times the LD50 of our White Leghorn hens.

It is interesting that the dose level eliciting OPIDN was apparently lower in the hybrid hens than in the White Leghorn hens, raising the possibility that sensitivity to OPIDN was different in the two strains we studied. Dr. Cisson in our laboratory previously showed that another strain, the scaleless chicken, was less sensitive to TOCP than a New Hampshire line (Cisson and Wilson,1982), probably due to the rate at which TOCP was converted by the hen to its toxic metabolites. The commercial White Leghorn type chickens used for OP testing in the US are not a standard genetically homogeneous breed of chicken; every breeder develops their own strains and alters their genetics each year to improve performance. Such genetic differences may be involved in the discrepancy in LD50 values between our findings and those of Mobay and the fact that they found no symptoms of OPIDN in birds at 75 mg/kg.

The levels of antidotes used in the study were established for the commercial White Leghorn hens and later applied to the hybrid chickens in which some signs of a mild leg weakness were noted. Regardless, there was no inhibition of NTE or lesions in the nerves of antidote-treated hybrid hens, similar symptoms of OPIDN appeared in the White Leghorn birds, where there was

no indication of an effect of the antidotes themselves, indicating that any symptoms of leg weakness shown by the hybrid hen controls were unrelated to those caused by the OP. (It is well known that the antidotes themselves are toxic to animals when given in the absence of the poison they are to counteract).

Inhibition of NTE in the brain of chickens treated with an OP has been shown to be a reliable indicator of the potential neurotoxicity of the agent (Johnson, 1982). Inhibitions greater than 70% usually result in symptoms of the neuropathy several weeks later. In this case, 100 mg/kg of IFP caused a 75 to 85% inhibition of brain NTE in hybrid chickens. The inhibition of NTE of the Leghorns (data not shown) was 64% at 100 mg/kg one day after treatment. No inhibition of NTE was noted in antidote injected controls of either strain.

The interim findings presented here should alleviate some of the concerns about IFP and antidotes; rats and chickens were both protected by the antidotes licensed for use in the US. However, the protection given to rats was not great, and some animals died days after exposure. The response of the rats may be a "worst case" study; they are noted for not responding well to such agents. The next step is to examine the action of the antidotes on another mammal, the guinea pig.

The finding that IFP causes OPIDN in the chicken raises a different set of toxicological concerns. There is no general agreement on the use of neuropathic OPs. One of them, TOCP (a plasticizer) has been responsible for the paralysis of many thousands of people since the turn of the century. Others like EPN, have been used widely in places like cotton fields without either great public outcry or attested cases of paralysis in humans. Consequently, toxicologists are not of a single mind about OPs that cause OPIDN; some favor banning all of them, others assess their risk after determining no-effect-levels.

Considering the extensive testing required before an OP is licensed, it is appropriate to wonder why IFP was not recognized as neuropathic by others. Part of the answer is that EPA regulations do not require that OPs be tested at their highest protected levels. Unfortunately, the fact that the IFP induced neuropathy appeared only at high dose levels need not mean that there is little danger of contracting the disorder; OPIDN is known to occur with low level repeated exposures to neuropathic OPs. For example, Abou-Donia and Graham (1978, 1979) found that although EPN caused OPIDN in hens at an acute single dose of 25 mg/kg, its no-effect-level for repeated exposure was between 0.1 and 0.01 mg/kg, more than 100 fold less. What is needed is a study of the lowest dosage level of IFP that will cause the neuropathy after repeated doses. (EPA regulations require that 90 day chronic trials be carried out on all OPs known to cause OPIDN to establish reasonable no-effect-levels.)

Unlike the acute cholinergic toxicity of OPs, there is neither cure nor approved antidote for OPIDN. Immediate treatment of experimental animals with carbamates (Johnson and Lauwerys, 1969) glucocorticoids (Baker et al, 1982) and other chemicals have been reported to block its occurrence. However, the insidious onset of OPIDN probably precludes the effective use of such treatments in humans, and I know of no case where such treatments have been given clinically. Although severe damage to the nervous system (Stage 4) of OPIDN is usually not reversible, animals with less intense systems may slowly regain use of their limbs. For example, a White Leghorn hen exposed to 100 mg/kg of IFP by gavage that became unable to rise off its hocks (Stage 3) three weeks after treatment, has recovered the use of its lower limbs several months after exposure.

CLOSING COMMENT

There are several toxicities to be considered in assessing the risks in applying IFP. One is the matter of the antidotes; the other of the delayed neuropathy. Although I dislike publishing results on the front page of a newspaper before they are ready to present to the scientific community, I felt it of the utmost importance to warn the CDFA about the symptoms of the neuropathy as soon as they were noted and I was pleased when Assistant Director McGee promptly announced the preliminary findings to the press. The results of the studies completed this month fully support the statements made there by Director McGee; IFP poisoning responds to antidotes and, at high acute doses, causes specific nerve damage.

It is always a temptation to automatically cry doom when a chemical used in the home, workplace or the environment is shown to have unforseen toxic effects. In this case, I think it most unlikely that the few applications that were done in Orangevale created a risk to anyone involved of contracting OPIDN. Exposure levels to humans could not have been above the microgram levels, far below the doses we used in this study. Rational assessment of the risks involved in the continued use of IFP must wait upon the results of future studies such as one in which experimental animals are repeatedly exposed to low levels of the agent.

COLLEAGUES

Participants in the study are graduate student Michael Hooper, graduate student Ed Chow, and technician Craig Ackerman. Pathologist R.J. Higgins performs the histology and CDFA Staff Toxicologist J.B. Knaak is project officer.

Barry W. Wilson
Professor

REFERENCES

Abou-donia, M.B. and Graham, D.G. Delayed neurotoxicity of O-ethyl-O 4-nitrophenyl phenylphosphonothioate: toxic effects of a single oral dose on the nervous system of hens. Tox. Appl. Pharm. 48:57-66 (1979).

Abou-Donia, M.B. and Graham, D.G. Delayed neurotoxicity of O-ethyl-O 4-nitrophenyl phenylphosphonothioate: subchronic (90 days) oral administration in hens. Tox. Appl. Pharm. 45:685-700 (1978).

Baker, T., Drakontides, A.B. and Riker, W.F. Jr. Prevention of the organophosphorus neuropathy by glucocorticoids. Exp. Neurol. 78:397-408 (1982).

Cisson, C.M. and Wilson, B.W. Percutaneous toxicity and the delayed neurotoxicity of organophosphates in the scaleless hen. Tox. Appl. Pharm. 67 :310-321 (1983).

Ishikawa,Y., Chow,E, McNamee, M.G., McChesney,M. and Wilson, B.W. Separation of paraoxon and mipafox sensitive esterases by sucrose density gradient sedimentation. Tox. Letters 17:315-320 (1983).

Johnson, M.K. and Lauwerys, R. Protection by some carbamates against the delayed neurotoxic effects of di-isopropyl phosphorofluoridate. Nature 222:1066 (1969).

Johnson, M.K. The target for initiation of delayed neurotoxicity by organophosphorus esters: biochemical studies and toxicological applications. Reviews in Biochem. Toxicol. 4:141 (1982).

Pesticide Residues In Food: 1981 Evaluations. FAO Plant Production and Protection Paper # 42. 1982.

TABLE 1
Schedule of Experiments

August-September	Inquiries, planning, design of experiments.
Early October	Pilot studies on chicken
Late October	LD50 and antidote studies on chicken; recognition of OPIDN symptoms.
Early November	LD50 studies on rats; begin confirmatory OPIDN studies in hybrid chickens. Confer with CDFA.
Late November	Antidote studies on rats; OPIDN studies on White Leghorn chickens.
Early December	Antidote studies on rats; complete chicken studies.
Late December	Compile data, prepare report; plan studies on guinea pigs and AChE.

TABLE 2
DISPOSITION OF BIRDS

Experiment	Objective	Route	Dose	Ant	Birds(Number)
1010	Pilot Antidote	SC	25-50	Y	Hybrid (2)
1011	Pilot LD50	G	13-30	N	Leghorn (3)
1011	Pilot High Dose	SC	100	Y	Hybrid (1)
1013	Pilot LD50	G	15-50	N	Leghorn (4)
1013	Pilot LD50	SC	15-50	N	Hybrid (4)
1019	Pilot LD50	G	8-18	N	Leghorn (3)
1020	Pilot LD50	G	3-7	N	Leghorn (3)
1020	Antidote	SC	50	Y	Hybrid (3)
1024	LD50	G	1.5-6.75	N	Leghorn (24)
1026	High Dose	G	100	Y	Leghorn (5)
1026	High Dose	SC	100	Y	Hybrid (5)
1101	Antidote	G	25-75	Y	Leghorn (15)
1104	OPIDN	SC	100 200(TOCP)	Y	Hybrid (5)
1114	OPIDN/NTE	SC	100 200(TOCP)	Y	Hybrid (12)
1115	OPIDN	SC	100	Y	Hybrid (6)
1119	OPIDN	SC	100 200(TOCP)	Y	Leghorn (9)
1207	OPIDN	SC	100-150 200(TOCP)	Y	Leghorn (10)

Disposition of White Leghorn Hyline and UCD hybrid hens. Dose refers to IFP in mg/kg unless otherwise indicated. Ant refers to use of antidotes atropine and 2-PAM. TOCP is tri-ortho cresyl phosphate, a neuropathic OP.

TABLE 3
DOSE/RESPONSE TO IFP BY HENS

Dose	Dead	Total	Percent
7-20	6	6	100
5-6.75*	5	6	83
4.5	0	5	0
3.0	0	6	0
1.5	0	5	0
0	0	4	0

Dose in mg/kg body weight. Mortality after 48 hours. * 1/1 at 5.0; 4/5 at 6.75 mg/kg. IFP in PEG 400 delivered by gavage to fasted White Leghorn hens. Compiled from 5 experiments.

TABLE 4
DOSE/RESPONSE TO IFP BY RATS

Dose	Dead	Total	Percent
80	4	4	100
55	5	5	100
50	4	4	100
45	3	5	60
40	10	18	56
35	1	5	20
30	0	5	0
25	0	5	0
20	0	5	0

Dose in mg/kg body weight. Mortality at 96 hours. IFP administered in PEG 400 to fasted animals. Compiled from 4 experiments.

TABLE 5
ANTIDOTES FOR HENS AND RATS

HENS

I. STOCK SOLUTIONS

OFT 100 mg Oftanol/ml in PEG 400.
ATR 40 mg/ml atropine sulphate in dist. water
PAM 100 mg/ml 2-PAMCl in dist. water

II. PROPHYLACTIC S.C. TREATMENT

Time Intervals		OP and drugs	Comments
DAY	Clock	Elapsed	Injection	
1	10:30 AM	-30 min	20 mg/kg ATR	full dose before
	11 AM	0	100 mg/kg OFT	high dose
	11:30 AM	+30 min	50 mg/kg PAM	full dose after
	6 PM	7 hrs	10 mg/kg ATR	1/2 doses
			25 mg/kg PAM	
			OVERNIGHT	
2	6-7 AM	19 hrs	10 mg/kg ATR	1/2 doses

			25 mg/kg PAM	
	6-7 PM	31 hrs	5 mg/kg ATR	1/4 doses
			12.5 mg/kg PAM	
			OVERNIGHT	
3	6-7 AM	43 hrs	5 mg/kg ATR	1/4 doses
			12.5 mg/kg PAM	
	6-7 PM	55 hrs	5 mg/kg ATR	1/4 doses
			12.5 mg/kg PAM	
			OVERNIGHT	
4	6-7 AM	67 hrs	5 mg/kg ATR	1/4 doses
			12.5 mg/kg PAM	
	6-7 PM		OBSERVE	0 doses

III. THERAPEUTIC GAVAGE TREATMENT

DAY	Time Intervals Clock	Elapsed	OP and drugs Injection	Comments
1				
	11 AM	0	100 mg/kg OFT	high dose
	11:20-30	20-30 min	20 mg/kg ATR	full doses after
			50 mg/kg PAM	
	6 PM	7 hrs	10 mg/kg ATR	1/2 doses
			25 mg/kg PAM	OMIT FOR OFT <100
			OVERNIGHT	
2	6-7 AM	19 hrs	10 mg/kg ATR	1/2 doses
			25 mg/kg PAM	
	5 PM	30 hrs	10 mg/kg ATR	1/2 doses
			25 mg/kg PAM	STOP OFT 25
			OVERNIGHT	
3	6-7 AM	43 hrs	5 mg/kg ATR	1/4 doses
			12.5 mg/kg PAM	
	6-7 PM	55 hrs	5 mg/kg ATR	1/4 doses
			12.5 mg/kg PAM	
			OVERNIGHT	
4	6-7 AM	67 hrs	5 mg/kg ATR	1/4 doses
			12.5 mg/kg PAM	
	6-7 PM		OBSERVE	0 doses

RAT GAVAGE

I. STOCK SOLUTIONS FOR 280 GRAM RATS

ATR 112 mg/ml for 0.25 ml full dose
PAM 56 mg/ml for 0.25 ml full dose

II. THERAPEUTIC GAVAGE

DAY	Time Intervals Clock	Elapsed	OP and drugs Injection	Comments
1	10:30 AM	0	OFT doses	OFT in PEG 400
	11 AM	+30 min	0.25 ml ATR	full doses
			0.25 ml PAM	
	4 PM	5 hr	0.25 ml ATR	full doses
			0.25 ml PAM	
	10 PM	11 hr	0.25 ml ATR	full doses
			0.25 ml PAM	
			OVERNIGHT	
	7 AM	20 hr	0.15 ml ATR	1/2 doses
			0.15 ml PAM	

Noon	25 hr	0.15 ml ATR 0.15 ml PAM	1/2 doses
6 PM	31 hr	0.15 ml ATR 0.15 ml PAM	1/2 doses
Midnight	37 hr	0.15 ml ATR 0.15 ml PAM	1/2 doses
7 AM	44 hr	0.15 ml ATR 0.15 ml PAM	1/2 doses
Noon		OBSERVE	
Evening		OBSERVE	

TABLE 6
MORTALITY OF HENS FROM IFP AFTER ANTIDOTES-

Dose	Antidotes	Dead	Total
100	85 138P	3	5
75	75A 188P	1	5
50	75A 188P	2	5
250	30A 75P	0	5

A is total dose of atropine; P is total dose of 2-PAM. White Leghorn hens. Mortality to 6 days;. Antidote schedule as in Table 5 Compilation of two experiments.

TABLE 7
MORTALITY OF RATS FROM IFP AFTER ANTIDOTES

Dose	Dead	Total
135	3	6
90	4	5
80	1	4
45	1	5
40	0	4

Mortality to 6 days. Compilation of 2 experiments. 135, 90 and 45 mg/kg given injections of 100 mg/kg atropine and 50 mg/kg 2-PAM 2X a day for 3 days. Last shot was atropine only; 45 mg/kg received 1/2 dose instead of full one time. 80 and 40 mg/kg received 3 full doses first day, 4 1/2 doses the second day and one 1/2 dose on the third day.

TABLE 8
SYMPTOMS OF OPIDN IN HYBRID HENS

Stage	Days To Most Extreme Stage
Two	36
Three	13 14 15 36
Four	13 16 17 20 28 17 20

Each value is days for a single hen to reach the stage indicated. Stage 2 Stumbles; Stage 3: Can't stand; Stage 4: Paralyzed.

TABLE 9
NTE LEVELS
HYBRID CHICKENS

Day	Treatment	Total	NTE
1	None	32.3	6.31
	Control	36.8	6.35
	IFP	23.0	1.72
		21.9	1.32
3	None	34.7	5.04
	Control	39.2	7.08
	IFP	24.5	1.05
		20.7	0.80
7	None	48.3	6.53
	Control	42.4	7.47
	IFP	24.3	1.83
		26.4	1.89

Units are Absorbance/ min/g of brain; 5 month old hybrid crosses. None: uninjected hens. Control: hens injected with atropine, 2-PAM and PEG. IFP: 100 mg/kg isofenphos/ kg, s.c. Each value is the average of two (IFP) or three (None, Control) replicates. Total is the total hydrolysis of phenyl valerate; NTE is that part of the hydrolysis due to the addition of mipafox to a paraoxon-treated homogenate.