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Effects of Sulprofos and Its Sulfoxide and Sulfone Metabolites on Laying Hens Fed the Compounds in the Diet

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The organophosphate insecticide sulprofos (Bolstar, BAY NTN 9306, *O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate) and its sulfoxide and sulfone metabolites were added to the diet of White Leghorn laying hens as a 2:5:3 mixture of sulprofos/sulfoxide/sulfone. At the highest treatment level (150 ppm), the treated diet was apparently highly unpalatable, and feed consumption was greatly reduced; loss of weight and drop in egg production resulted. At treatment levels of 50 ppm or lower in the diet, these effects were reduced or nonexistent. Serum cholinesterase (ChE) activities were significantly decreased in birds fed diets containing as little as 2.7 ppm of the insecticide mixture, but ChE activity increased quickly after the birds were transferred to untreated feed. Analysis of tissues and eggs from birds maintained on the treated diet for 28 days showed no detectable residues (<0.05 ppm) of sulprofos or its intact ester metabolites, except in skin and fat of some of the birds fed at the highest treatment level. Histopathological examination of selected tissues from the treated birds showed no lesions.

The organic phosphate insecticide sulprofos (*O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate, Bolstar, BAY NTN 9306 of Mobay Chemical Corp., Kansas City, MO) shows high insecticidal activity against certain phytophagous insects, particularly insecticide-resistant strains of *Heliothis* sp., yet its mammalian toxicity is well below that of many of the organic phosphate insecticides in current use. We have previously reported on the environmental behavior of sulprofos, including its photochemistry (Ivie and Bull, 1976), its fate in cotton plants and soil (Bull et al., 1976), and its metabolic behavior in laboratory rats (Bull and Ivie, 1976) and a lactating cow (Ivie et al., 1976). Among other findings, these studies revealed that the sulfoxide and sulfone analogues of sulprofos are two of its major environmental and metabolic transformation products. In the current studies, a mixture of sulprofos, sulprofos sulfoxide, and sulprofos sulfone was added to the diets of laying hens to permit evaluation of the interactions of these compounds with the birds and to determine the potential for appearance of residues in meat and eggs.

MATERIALS AND METHODS

Chemicals. The chemicals used for treatment of the birds were analytical standard grade samples of sulprofos and its sulfoxide (*O*-ethyl *O*-[4 -(methylsulfinyl)phenyl] *S*-propyl phosphorodithioate) and sulfone (*O*-ethyl *O*-[4-(methylsulfonyl)phenyl] *S*-propyl phosphorodithioate) derivatives. Additional compounds used in the residue analysis phase of the study included sulprofos oxygen analogue (*O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorothioate), the oxygen analogue sulfoxide (*O*-ethyl *O*-4-[methylsulfinyl)phenyl] *S*-propyl phosphorothioate), and the oxygen analogue sulfone (*O*-ethyl *O*-[4-(methylsulfonyl)phenyl] *S*-propyl phosphorothioate. Each of these chemicals was supplied by the Mobay Chemical Corp.

Treatment. This study was made up of two parts, experiment I and experiment II. In experiment I, 34-week-old White Leghorn hens (Ideal Poultry Co., Cameron, TX) that were in full egg production were used. Thirty hens were randomly divided into five groups of six birds each but were housed in individual laying cages. Water was available at all times. Two hundred grams of treated feed was placed in the feed trough before each bird, and the feed consumed each day was measured by weighing the amount of residual feed in the trough. This was then discarded and was replaced with 200 g of fresh treated feed.

The five groups were provided feed containing the following levels of sulprofos/sulfoxide/sulfone mixture: 0, 5, 15, 50, and 150 ppm; the chemical mixture consisted of 20% sulprofos, 50% sulfoxide, and 30% sulfone. This ratio of sulprofos/sulfoxide/sulfone was chosen because it approximates the relative concentrations of these three products seen as residues in sulprofos-treated crops that are used in the manufacture of poultry feeds (Flint, 1978). To minimize the possibility of chemical degradation of the insecticide mixture in the treated feed, it was freshly prepared each week, and the feed samples were stored frozen until immediately before they were placed before the birds.

The feed for each treatment level was prepared in 9.0-kg batches to provide sufficient feed for each group of birds for 1 week (200 g bird^{-1} day^{-1}). Preparation of feed for the 5 ppm treatment level is described below as an example of the mixing procedure. Sulprofos (9.0 mg), sulfoxide (22.5 mg), and sulfone (13.5 mg) were dissolved in 15 mL

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of ethanol, and this solution was mixed with 180 g of corn oil. The ethanol–corn oil solution was then mixed thoroughly with 8.82 kg of Purina Laying Crumbles (16% protein) in a Blakeslee Model F-30T mixer (Blakeslee and Co., Chicago, IL). Thus, 45.0 mg of a 2:5:3 mixture of sulprofos/sulfoxide/sulfone was mixed with 9.0 kg of feed to yield the 5 ppm treatment feed, containing 2% by weight of added corn oil. The treated feed was then weighed in individual plastic bags for daily distribution to the birds, and the bags were held frozen until just before they were used.

The treated feed for the 15, 50, and 150 ppm treatment groups was prepared similarly. Feed for the control group was prepared by mixing with the feed the ethanol–corn mixture only.

Sampling and Measurements. Each bird was individually weighed at the beginning and at the end of the experiment. Feed consumption was determined daily but is reported as the average for an entire week. Egg production was recorded daily.

Blood pseudocholinesterase (ChE) levels were determined pretreatment and at 2 and 4 weeks after treatment. Blood samples were collected from a wing vein into nonheparinized tubes. After the blood clotted, it was centrifuged, and the serum was collected and analyzed for ChE activity with a Model 368 Clinicard Analyzer (Instrumentation Laboratory, Lexington, MA). The required reagents and materials were obtained as "pseudocholinesterase sets" (Item 32307, Harleco, Gibbstown, NJ). The method is a modification of that developed by Ellman et al. (1961). Cholinesterase hydrolyzes acetylthiocholine-forming thiocholine, which then hydrolyzes the sulfur bonds of 5,5′-dithiobis(2-nitrobenzoic acid) to form 2-nitro-5-mercaptobenzoic acid. Cholinesterase activity is determined kinetically by the rate of increase of 2-nitro-5-mercaptobenzoic acid measured at 455 nm.

After the birds had been maintained on the treated diets for 4 weeks, three or four birds from each group were killed, and tissue samples were collected and frozen at −70 °C for later residue analysis. The other birds in each group were then provided a control diet containing no sulprofos or metabolites. Cholinesterase was measured at 1 and 2 weeks during this withdrawal period.

Because the lowest treatment level (5 ppm) significantly inhibited serum ChE activity (discussed in the Results section), an additional study, experiment II, was undertaken to determine the level of sulprofos–sulfoxide–sulfone in the diet at which serum ChE would not be inhibited. Twelve hens of the same strain and age used in experiment I were divided into two groups of six birds each and were held in laying cages as in the first study. One group was fed untreated feed, and the other group a diet containing the 2:5:3 mixture of sulprofos/sulfoxide/sulfone. The six birds in the treatment group received a 0.1-ppm diet during the first week, and the treatment level was successively increased each week to 0.3, 0.9, and 2.7 ppm during the next 3 weeks. The birds were transferred to untreated feed during the fifth and final week of the study. Serum ChE activity was determined pretreatment and at the end of each week. No other data were recorded, and residues in tissues or eggs from the birds were not analyzed.

Analysis of Residues in Tissue and Eggs. The basic gas–liquid chromatographic (GLC) procedure used in this study was one developed in the laboratories of the Mobay Chemical Corp. for the analysis of sulprofos and its metabolites in animal tissue (Sandie and Gronberg, 1975). The extraction and clean-up procedure was used without modification in the poultry tissue analysis reported herein, but certain modifications were required in adapting the procedure for analysis of whole egg samples. In the analysis, all residues of sulprofos and its intact ester metabolites (Table I) were oxidized to sulprofos oxygen analogue sulfone, which was then analyzed directly by GLC.

Table I. Validation of the Gas–Liquid Chromatographic Method for Determination of Sulprofos and Its Metabolites in Eggs and Tissues of Chickens[a]

	recovery of added compound, %[b,c]					
tissue	PSS	PSSO	$PSSO_2$	PO	POSO	$POSO_2$
giblets	116	70	111	134	81	96
muscle	80				98	
skin	90				95	
fat	88				96	
egg	78	84	83	94	80	115

[a] Samples fortified at the 0.05-ppm level. [b] Measured as the sulprofos oxygen analogue sulfone. [c] Abbreviations are as follows: PSS, sulprofos; PSSO, sulprofos sulfoxide; $PSSO_2$, sulprofos sulfone; PO, sulprofos oxygen analogue; POSO, sulprofos oxygen analogue sulfoxide; $POSO_2$, sulprofos oxygen analogue sulfone.

Extraction and Cleanup. Tissue (25 g) or egg (50 g) samples were homogenized with either 200 mL (tissue) or 300 mL (egg) of acetonitrile for 3 min at high speed in a Waring blender. The homogenate was then filtered by vacuum through Whatman No. 1 filter paper into a 1000-mL side-arm flask. The blender was washed repeatedly with a total of 100 mL of additional acetonitrile, which was then used to wash the filter cake. The filter cake was then returned to the blender and homogenized with either 200 mL (tissue) or 300 mL (eggs) of hexane for 3 min. The homogenate was then filtered by vacuum into the side-arm flask containing the acetonitrile extract. The 100-mL hexane extraction of the cake was repeated. The total extracts (acetonitrile and hexane) were refiltered, transferred to a 1000-mL separatory funnel, and then shaken for 30 s. After the layers separated, the acetonitrile was drained into another separatory funnel that contained 100 mL of hexane. The hexane in the first separatory funnel was rewashed with 100 mL of acetonitrile; the acetonitrile was added to the contents of the second separatory funnel. After the combined acetonitrile extracts and washes were shaken for 30 s, the acetonitrile was drained into a 1000-mL round-bottomed flask which was then attached to a vacuum rotary evaporator. The hexane extracts and washes were discarded, and the acetonitrile was removed by evaporation to dryness. For eggs only, the residual material was dissolved in 50 mL of water, transferred to a separatory funnel, and extracted three times with 50-mL portions of benzene. After each extraction, the layers were separated by centrifugation. The benzene extracts were combined in a 500-mL round-bottomed flask, and the solvent was removed with a vacuum rotary evaportor. The benzene/water partition of egg extracts was necessary to remove certain unidentified materials extracted from eggs which interfered with the subsequent oxidation procedure. Subsequent cleanup and analysis of extracts were identical for eggs and tissue.

The residue remaining in the flask after evaporation of the acetonitrile (tissue) or benzene (eggs) was transferred to a 250-mL separatory funnel by repeated washes with a total of 100 mL of chloroform, then 25 mL of water, then 75 mL of acetone. The chloroform/water/acetone mixture was shaken for 30 s, and the layers were allowed to separate. The lower phase (chloroform) was drained through a glass column containing anhydrous sodium sulfate into a 500-mL round-bottomed flask. The sodium sulfate was

rinsed with an additional 25 mL of chloroform. The flask containing the chloroform extract was then attached to the rotary evaporator, and the solvent was removed by evaporation.

Oxidation and Column Chromatography. An elution column was prepared as follows: silica gel (Fisher silica gel S-679, 100–200 mesh) was first activated at 130 °C for 16 h then deactivated by addition of 50 mL of water dropwise to 450 g of silica gel. The deactivated gel was thoroughly mixed and then allowed to stand overnight in a sealed container. A benzene slurry of 10 g of the deactivated gel was added to a 10 × 300 mm glass column fabricated with a sealed-in coarse porosity fritted disc and a Teflon stopcock (Corning No. 445721). After the benzene had drained out of the column and the silica gel had settled, 5 g of sodium sulfate and a glass-wool plug were added sequentially to the top of the column.

The residue from the chloroform–water–acetone extraction was dissolved in 19 mL of dichloromethane. To this solution was added 1 mL of the oxidation reagent (5% *m*-chloroperoxybenzoic acid (MCPBA) in dichloromethane). The solution was maintained at room temperature and was swirled several times during the 17-min reaction period. This treatment was sufficient to completely oxidize the parent compound and each of its potential phosphate ester metabolites (Table I) to the oxygen analogue sulfone ($POSO_2$) state.

After the oxidation was completed (17 min), the solution was transferred to the silica gel column that was attached to a 1000-mL vacuum flask containing 25 mL of a 0.25% sodium bisulfite solution in methanol. The solvent was caused by mild vacuum to drain to, but not past, the top of the sodium sulfate layer. The contents of the reaction flasks were then quantitatively transferred to the column sequentially with 30 mL of dichloromethane and then 400 mL of benzene and rapidly pulled through the column by vacuum. The benzene wash of the silica gel column after addition of the oxidation reaction mixture was necessary in order to rapidly remove any unreacted MCPBA from the column. If the MCPBA was not washed off but was allowed to concentrate on the column, recovery of the $POSO_2$ was greatly reduced. This loss was probably due to oxidative degradation of the $POSO_2$ on the column. As the last of the benzene reached the top of the sodium sulfate, the vacuum was released, and the flow of solvent stopped. The vacuum flask was replaced by a 250-mL round-bottomed flask. The $POSO_2$ product was eluted with 150 mL of acetonitrile/benzene (1:9) by gravity flow at a rate of approximately 1 mL/min. After the elution from the silica gel was completed, the flask was attached to the rotary evaporator, and the solvent was removed by evaporation to dryness under vacuum.

Gas Chromatographic Analysis. A Tracor MT-220 gas chromatograph (Tracor Inc., Austin, TX) with a flame photometric detector (FPD) and a phosphorus filter (526 nm) was used for residue analysis. The signal from the detector was fed into an Autolab System IV computing integrator (Spectra-Physics, Mountain View, CA) as well as a Westronics 1-mV strip chart recorder (Westronics, Inc. Fort Worth, TX). The gas chromatograph was fitted with a multiple inlet and a gas-flow switching system that allowed alternate selection of two columns with the same detector. By use of the switching valves, the effluent from either of the two columns could be directed through the FPD. In this study, the valve system served two functions: (1) upon injection, the effluent from the primary or analytical column was vented into the atmosphere and that from the secondary or confirmatory column was directed through the FPD (this system prevented the solvent from extinguishing the detector flame); and (2) the instantaneous use of a confirmatory column without necessitating removal of the primary column was allowed.

The primary column was a 0.6 m × 3 mm i.d. glass tube packed with 3% OV-17 on Gas-Chrom Q (80-100 mesh). The confirmatory column was a 0.4 m × 3 mm i.d. glass tube packed with 6% QF-1 on Chromosorb W (80–100 mesh). Both columns were installed for direct on-column injection and were operated at 255 °C isothermal; prepurified nitrogen (40 cm^3/min) was the carrier gas, and the inlet temperature was 230 °C. The FPD with phosphorus filter (526 nm) was operated with a detector temperature of 210 °C, hydrogen flow of 100 cm^3/min, and air flow of 15 cm^3/min. The detector attenuation was 8 × 10^2.

Elution times for the $POSO_2$ product were approximately 165 s for the primary column and 90 s for the confirmatory column, with no interfering peaks. An injection of 0.05 ng of $POSO_2$ resulted in a peak of about 5% of full scale above background. $POSO_2$ could be measured in an injection of up to 4 μL of 2 mL of acetone containing the extract from 25 g of tissue or 50 g of eggs.

The FPD with the 526-nm filter is designed to respond specifically to organophosphorus compounds. Because sulprofos and each of its potential phosphate ester metabolites were oxidized to $POSO_2$ and chromatographed as a single compound, only a single organophosphorus peak was observed on the chromatograms. The retention times and peak geometry of the peaks observed from either spiked control samples of extracts of test samples corresponded to the retention time and peak geometry of the peak produced after injection of an authentic standard of $POSO_2$. In addition, the identity of residues recovered from spike and test samples was confirmed by GLC with the confirmatory column.

The analytical method was validated at 0.05 ppm for sulprofos and its metabolites as indicated in Table I. Known amounts of the various compounds were added to control samples of eggs or tissues, and the spiked samples were extracted and analyzed as described previously in this section. Recoveries for the various compounds and tissues ranged from 70 to 134% (Table I). The identity of the sulprofos oxygen analogue sulfone recovered was confirmed by GLC on two columns.

Pathology. Samples of liver, kidney, brain, lung, spleen, and ovary were taken from two birds from the control and each of the four treatment groups at the end of the 28-day feeding period. The tissues were fixed in 10% phosphate-buffered formalin, routinely processed through an Autotechnicon tissue processor, paraffin embedded, sectioned at 5 μm, and stained with hemotoxylin and eosin for subsequent histopathological examination.

RESULTS

Weight Changes and Feed Consumption. During the 28-day feeding period, the birds fed the 150-ppm diet lost about 27% of their initial body weight, those fed the 50-ppm diet lost about 11%, and those fed the 15-ppm diet lost about 4% per bird (Table II). During the 28-day feeding period, the control birds and those fed the 5-ppm diet gained about 1 to 2%. Examination of the feed consumption data (Table III) clearly indicated that the weight loss experienced by some of the birds was related to greatly reduced feed consumption. The addition of sulprofos and its metabolites to the feed apparently decreased the palatability of the feed to the birds. Feed consumption dropped by two-thirds in the 150-ppm diet group and was reduced to a lesser degree in the 50-ppm

Table II. Body Weight of Laying Hens Fed Sulprofos and Its Sulfoxide and Sulfone Metabolites in the Diet for 28 Days[a]

treatment level, ppm	hen weight and weight change during a 4-week treatment period: pretreatment weights,[b] g ± SD	after 28 days of treatment: weights, g ± SD	after 28 days of treatment: weight change, % of pretreatment ± SD[c]
0	1647 ± 323	1677 ± 389	+1.3 ± 4.4
5	1550 ± 217	1587 ± 264	+2.1 ± 4.3
15	1699 ± 239	1624 ± 205	-4.2 ± 3.8
50	1559 ± 318	1452 ± 342	-11.2 ± 8.4[d]
150	1592 ± 248	1177 ± 165	-27.1 ± 11.8[d]

[a] Experiment I. [b] Body weight immediately before initiation of treatment. [c] (+) overall average weight gain; (-) overall average weight loss. [d] Differs significantly from control group at $\alpha \leq 0.05$ according to Tukey's ω-procedure (Steele and Torrie, 1960).

diet group. In all groups in which feed consumption was affected, the decreased consumption was observed within the first week of the treatment period and remained relatively constant throughout the rest of the period.

Egg Production. Egg production by hens fed the treated diets was affected only in the two highest treatment groups, 50 and 150 ppm (Table IV). In the 150-ppm diet group, the production of eggs was reduced by 70–75% from pretreatment production.

Cholinesterase Inhibition. Consumption of rations containing 5 ppm or more of sulprofos–sulfoxide–sulfone rapidly and dramatically depressed serum ChE activity (Table V). At the first sampling 14 days after treatment began, ChE activity was depressed by about 80% in the 5-ppm treatment group and >95% in the higher treatment groups. However, when the birds were transferred to untreated feed, serum ChE activity recovered quickly (Table V). Although the data collected during the withdrawal period are based on results with only 1 or 2 birds, ChE activity clearly had recovered to essentially normal values within 7 days. Serum ChE data from a subsequent feeding study with lower levels of the sulprofos–sulfoxide–sulfone mixture (experiment II) are shown in Figure 1. The depression of ChE activity was first clearly indicated at the end of the third week, during which the birds had been fed the 0.9-ppm diet. Cholinesterase activity at this time was depressed by about 20% from pretreatment levels. At the end of the fourth week, after the birds had been fed the 2.7-ppm diet, the depression of ChE activity was about 70%. As in experiment I, transferring the birds to control feed resulted in rapid recovery of serum ChE activity (Figure 1).

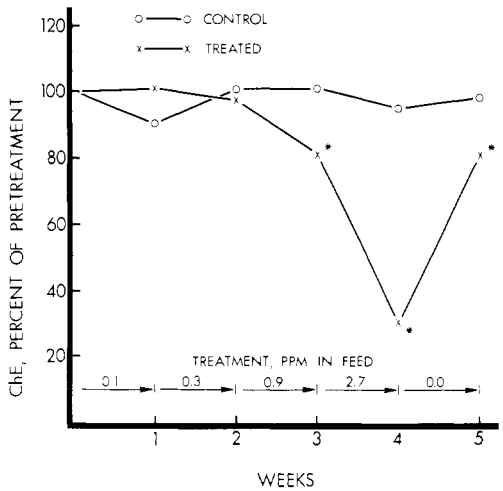

Figure 1. Serum cholinesterase activity in laying hens fed sulprofos and its sulfoxide and sulfone metabolites in the diet (experiment II). Asterisk indicates a significant difference from control group at $\alpha \leq 0.05$ (Student's t test).

Residue Analysis. Analysis of numerous tissue and egg samples collected during the 28-day study showed very little evidence of accumulation of sulprofos and its intact ester metabolites in the tissue or in eggs (Table VI). The fat and skin of birds in the highest treatment group contained low residues, but eggs and other tissues analyzed did not contain detectable residues (<0.05 ppm; Table VI).

Bird Health and Mortality. During the study, four of the birds died: one in the control group, one in the

Table III. Feed Consumption by Laying Hens Fed Sulprofos and Its Sulfoxide and Sulfone Metabolites in the Diet for 28 Days[a]

weeks on treatment	feed consumption, % of pretreatment ± SD,[b] at indicated treatment level, ppm[c]: 0	5	15	50	150
1	90.2 ± 8.3	99.4 ± 7.3	80.3 ± 7.9	69.1 ± 9.2[d]	28.7 ± 9.3[d]
2	91.5 ± 14.2	93.1 ± 15.7	82.0 ± 15.0	68.2 ± 11.6[d]	31.9 ± 5.7[d]
3	88.7 ± 6.7	94.1 ± 9.1	80.6 ± 9.7	70.2 ± 20.8	29.8 ± 9.1[d]
4	80.4 ± 14.9	85.5 ± 19.9	74.5 ± 13.9	65.8 ± 14.9	27.7 ± 6.5[d]

[a] Experiment I. [b] Average daily feed consumption during the week immediately before initiation of treatment. [c] Daily average for the entire week. [d] Differs significantly from control group at $\alpha \leq 0.05$ according to the Tukey's ω-procedure (Steele and Torrie, 1960).

Table IV. Egg Production by Laying Hens Fed Sulprofos and Its Sulfoxide and Sulfone Metabolites in the Diet for 28 Days[a]

weeks	(eggs hen^{-1} week^{-1}) ± SD at indicated treatment level, ppm[b]: 0	5	15	50	150
pretreatment[c]	6.00 ± 1.46	5.05 ± 1.47	4.94 ± 1.95	5.50 ± 1.20	5.67 ± 0.82
on treatment					
1	6.17 ± 0.98	5.67 ± 0.52	4.50 ± 2.43	5.00 ± 0.89	3.67 ± 1.37[d]
2	6.33 ± 0.82	5.00 ± 1.55	5.33 ± 0.82	4.33 ± 1.97[d]	1.67 ± 1.37[d]
3	5.83 ± 0.98	5.33 ± 0.52	4.33 ± 2.16	4.33 ± 0.52	1.33 ± 1.21[d]
4	6.50 ± 0.84	5.83 ± 0.98	5.33 ± 2.58	5.17 ± 1.17	1.67 ± 1.17[d]

[a] Experiment I. [b] Average egg production during indicated week. [c] Egg production during the 3 weeks immediately prior to treatment. [d] Differs significantly from control group at $\alpha \leq 0.05$ according to Tukey's ω-procedure (Steele and Torrie, 1960).

Table V. Serum Cholinesterase Activity in Laying Hens Fed Sulprofos and Its Sulfoxide and Sulfone Metabolites in the Diet for 28 Days[a]

weeks	serum cholinesterase activity at indicated treatment level, ppm[b]				
	0	5	15	50	150
on treatment					
2	104.9 ± 10.0	22.1 ± 5.8[c]	9.2 ± 7.5[c]	6.4 ± 2.9[c]	5.7 ± 4.2[c]
4	110.9 ± 12.7	21.1 ± 10.0[c]	8.2 ± 4.9[c]	7.2 ± 1.2[c]	3.1 ± 1.1[c]
off treatment[d]					
1	118.5[e]	83.8[e]	60.1[e]	121.5[e]	98.2[f]
2	131.4[f]	94.4[e]	71.0[e]	135.5[e]	121.0[f]

[a] Experiment I. [b] Mean values ± SD, expressed as percentage of pretreatment level. [c] Differs significantly from control group at $\alpha \leq 0.05$ according to Tukey's ω-procedure (Steele and Torrie, 1960). [d] Statistical significance not determined because of limited observations. [e] Average of two birds. [f] One bird.

Table VI. Residues in Laying Hens Fed Sulprofos and Its Sulfoxide and Sulfone Metabolites in the Diet for 28 Days[a]

tissue	$POSO_2$, ppm, at indicated treatment level, ppm[b,c]				
	0	5	15	50	150
giblets	<0.05	<0.05	<0.05	<0.05	<0.05
muscle	<0.05	<0.05	<0.05	<0.05	<0.05
skin	<0.05			<0.05	0.05
fat	<0.05			<0.05	0.22
eggs[e]	<0.05	<0.05	<0.05	<0.05	<0.05[f]

[a] Experiment I. [b] All samples collected after 28 days of continuous sulprofos-sulfoxide-sulfone dietary exposure. [c] Expressed as parts per million of sulprofos oxygen analogue sulfone. [d] Composite sample including heart, liver, and cleaned gizzard. [e] Eggs collected during days 12-16 and 27-28. [f] Last samples analyzed were collected during days 18-22 because of low egg production in this group during later stage of the study.

50-ppm treatment group, and two in the 150-ppm treatment group. Malnutrition clearly contributed to the deaths of the two birds in the 150-ppm treatment group, for the birds were severely emaciated. Egg production in these birds had ceased 1–2 weeks before death. Cause of death was not apparent in the other two birds, for both were in good egg production and appeared healthy until the date of death. None of the other birds showed any visible signs of organophosphate poisoning or other toxicological effects, but birds in the 150-ppm treatment group showed obvious signs of weakness due to their emaciated condition.

Pathology. Histopathological examination of selected tissues taken from the birds after the 28-day treatment period revealed no lesions other than an occasional liver showing a moderate to moderately severe fatty metamorphosis. However, the liver changes observed could not be correlated either with treatment or with dose. Of the three birds with liver changes, one each were from the control, the 5-ppm, and 15-ppm treatment groups.

DISCUSSION

Results in the studies reported here have shown that continuous dietary exposure of laying hens to a mixture of sulprofos and its sulfoxide and sulfone metabolites does not result in the accumulation of organophosphate esters in the tissues or their secretion into eggs. Only in those birds fed the 150-ppm diet were any tissue residues observed, and the extremely emaciated condition of the birds fed the 150-ppm diet makes the significance of these findings questionable. Because of much greater feed intake, the birds fed the 50-ppm diet actually consumed almost as much total sulprofos-metabolite mixture as did those fed the 150-ppm diet, yet no detectable residues were observed in any tissue from the 50-ppm birds. Thus, the extremely weakened condition of the birds fed the 150-ppm diet may well have resulted in abnormal metabolism or excretion of the ingested insecticide, leading to the residues observed in skin and fat.

Serum ChE in these hens was highly susceptible to inhibition by the dietary sulprofos-metabolite mixture. On the basis of data in Figure 1, levels as low as 1–2 ppm of this 2:5:3 mixture of sulprofos/sulfoxide/sulfone in the diet can be expected to decrease serum ChE activity appreciably. However, two points must be emphasized. First, after cessation of exposure to even high levels of the insecticde mixture, serum ChE activity recovered to near pretreatment values within 1 week. Second, no signs of organophosphate poisoning or other toxicological symptoms were observed even in birds in which serum ChE activity was >95% depressed, including birds fed at least 50 times higher dietary levels of the insecticide mixture than that required to produce significant serum ChE depression (Table V, Figure 1). Serum ChE thus appears to be a highly sensitive monitor of exposure of chickens to sulprofos and its toxic metabolites, and depression of this enzyme would likely occur long before toxic levels were encountered. In fact, our data strongly suggest that poultry will not voluntarily consume levels of sulprofos and its metabolites sufficient to induce toxicity because of an apparent high degree of unpalatability of heavily contaminated feed.

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