Fate of Diflubenzuron in the Stable Fly and House Fly

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The metabolic fate of [^{14}C]diflubenzuron (Dimilin, TH-6040, *N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide) was studied after topical application to adult stable flies and house flies. Four days after treatment, radiocarbon residues were detected both on the surface and internally in the treated flies and in untreated flies of the opposite sex held with the treated flies. From 35 to 50% of the radiocarbon applied to the flies was detected in the excreta. Diflubenzuron was quite resistant to metabolism by stable flies; >97% of the recovered radiocarbon was the parent compound. House flies metabolized about 10% of the dose. These insects metabolized diflubenzuron by cleavage between the carbonyl and amide groups and by several undefined mechanisms. Treated stable flies secreted up to 1% of the dose into the eggs as unmetabolized diflubenzuron, but eggs from house flies contained much lower residues. Secretion of diflubenzuron into the egg and resulting toxicity to the developing embryo probably accounts for the "sterility" observed in the adults of several insect species exposed to this insecticide.

When the adults of several insect species are exposed to diflubenzuron (Dimilin, TH-6040, *N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide), they lay eggs that fail to hatch (Moore and Taft, 1975; Grosscurt, 1976; Wright and Harris, 1976; Wright and Spates, 1976). The ability of diflubenzuron to prevent hatch of eggs from exposed adults may prove of considerable value in controlling some insects, including the boll weevil (Taft and Hopkins, 1975) and biting flies on livestock (Wright and Harris, 1976; Wright and Spates, 1976). Diflubenzuron sprayed directly onto cattle effectively reduces egg hatch from stable flies and horn flies feeding on the treated animals (Wright and Harris, 1976). It seems likely that absorption of diflubenzuron by the adult insects and subsequent transfer to the eggs accounts for the ovicidal effects observed, but to our knowledge such transfer has not been documented. The studies reported here, using radiocarbon-labeled diflubenzuron, were designed to evaluate the metabolic behavior of the insecticide in two species of Diptera and to determine the extent to which the topically applied compound is transferred to the eggs of these insects.

MATERIALS AND METHODS

Chemicals and Analytical Procedures. The [^{14}C]-diflubenzuron (17.4 mCi/mM, with essentially uniform labeling in the two rings) and analytical standards considered as possible metabolites, were the same as those used in ruminant metabolism studies with diflubenzuron (Ivie, 1978). Metabolites were characterized by thin-layer chromatographic (TLC) comparisons with the authentic standards. Identical chromatographic behavior of a ^{14}C metabolite with a standard in each of six solvent systems (Ivie, 1978) was considered sufficient evidence of metabolite identity. Liquid scintillation measurements, radioautography, and oxygen combustion procedures were performed in an identical manner as reported in the ruminant studies with diflubenzuron (Ivie, 1978).

Treatment and Sampling. Stable flies (*Stomoxys calcitrans*) and house flies (*Musca domestica*, Orlando regular strain, insecticide susceptible) were from colonies maintained at this laboratory. The radiocarbon-labeled diflubenzuron was dissolved in acetone to a concentration of 2 μg/μL. Virgin adult flies, 4 days after emergence from the pupal stage, were treated topically on the ventral side of the abdomen with 1 μL of the [^{14}C]diflubenzuron solution (2 μg). Ten flies of each sex were treated, and the treated flies of one sex were held in a cage with 10 flies of the opposite sex that were treated with 1 μL of acetone only. Thus, for both species of flies studied, there were two treatment groups: treated males plus untreated females, and treated females plus untreated males. Each experiment was replicated twice. For controls in egg hatchability studies, 10 flies of each sex were treated with 1 μL of acetone only.

The 20 flies in each treatment group were held in a cylindrical plastic holding cup (7.5 cm high × 10 cm diameter), with plastic screen covering each end. For easier collection of the excreta, the cages were lined on the sides and bottom with sections of filter paper. The stable flies were fed daily by placing pads soaked with citrated bovine blood onto the top screen. House flies were fed powdered milk in a 1-oz paper cup placed inside the cage and were given water through moistened pads placed on the top screen.

On the third and fourth days after treatment of stable flies and on the fourth day only after treatment of house flies, the females were stimulated to lay eggs through the open screen by inverting the cage onto a moist black cloth. The eggs were collected and counted, a part was frozen for subsequent analysis, and samples of at least 100 eggs from each treatment group and replicate were studied for hatchability and survival (Wright and Spates, 1972). At the end of the fourth day after treatment, the flies were killed by freezing, the sexes were separated, and the flies and holding cages were frozen for later analysis.

Extraction and Analysis. Groups of four flies of each sex, treatment group, and replicate were soaked in acetone for 30 min to remove surface radiocarbon residues. The flies were then transferred to tubes containing 10 mL each of pH 2 water and ethyl acetate and were homogenized thoroughly with a Willems polytron homogenizer. The samples were centrifuged as required to break emulsions, the ethyl acetate phase was removed, and the water–residue phase was reextraced three more times with ethyl acetate. Radiocarbon in the ethyl acetate extracts was quantitated by liquid scintillation counting, then the extracts were dried over sodium sulfate, concentrated, and analyzed by two-dimensional TLC [benzene–ether (5:1) in the first direction and hexane–ethyl acetate–methanol (2:2:1) in the second direction] (Figure 1). Radiocarbon still in the aqueous phase was quantitated by liquid

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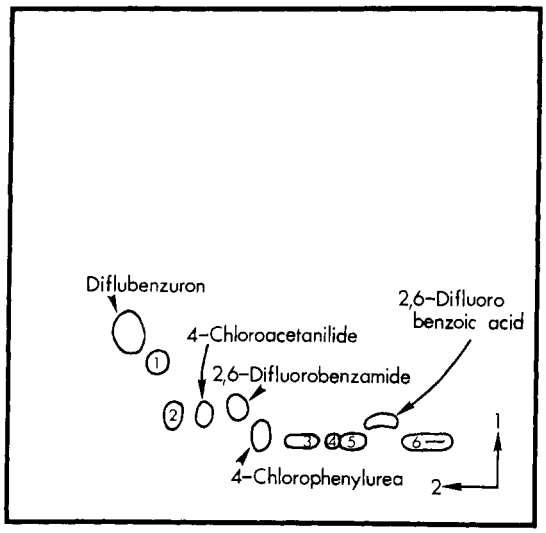

Figure 1. Representation of the TLC behavior of diflubenzuron metabolites in the stable fly and house fly. Numbers designate uncharacterized compounds. Plates were developed in benzene–ether (5:1) in the first direction followed by hexane–ethyl acetate–methanol (2:2:1) in the second direction.

scintillation counting, in the fly residue by oxygen combustion.

Radiocarbon residues in the excreta were extraced by homogenizing the paper from the lined cages in about 150 mL of pH 2 water with a Virtis "45" blade homogenizer. In the house fly studies, the feed cup and its residual powdered milk contents were extracted along with the cage lining. The paper–water slurry was then transferred to 250-mL centrifuge bottles, and the samples were extracted four times with ethyl acetate using the polytron homogenizer. After quantitation of radiocarbon in ethyl acetate, aqueous, and residue fractions by appropriate procedures, the ethyl acetate fractions were analyzed by TLC.

Samples of 100 eggs from each treatment group were soaked in 10 mL of acetone for 5 min to recover surface radiocarbon, then the samples were extracted by polytron homogenization in 10 mL of acetone. After centrifugation to sediment the very low amounts of residue, the extract was pipetted off, and the residue was extracted once again in an identical manner. Radiocarbon in the surface washes and egg extracts was quantitated and then analyzed by TLC; radiocarbon not extractable from the egg residue was quantitated by oxygen combustion.

The extent of transfer of radiocarbon from the treated flies to the feeding or watering pads was quantitated by combustion analysis. In addition, the entire holding cages were rinsed thoroughly with acetone to quantitate radiocarbon residues that penetrated the paper lining or were transferred to the uncovered screen at the top of the cage. The acetone rinse was highly destructive to the plastic and became gummy upon concentration, but this did not prevent studies on the nature of radiocarbon residues in this fraction by TLC.

RESULTS

Absorption, Excretion, and Transfer. Sample analysis 4 days after topical treatment of the flies with [^{14}C]diflubenzuron indicated that the compound is absorbed and subsequently excreted by both stable flies and house flies (Table I). The percentage of the applied [^{14}C]diflubenzuron remaining in and on the flies 4 days after treatment ranged from about 50% in the treated male stable flies to only about 20% in the treated female house flies. In all tests, at least 80% of the radiocarbon remaining in the treated flies was recovered in the acetone surface wash, and the rest was readily extractable from the insects–only trace residues could not be recovered from the insect debris (Table I). The excreta contained 35 to 50% of the applied radiocarbon. In stable flies, >98% of this was extractable with ethyl acetate, but in house flies only about 90% of the radioactivity in the excreta was extractable. Most of the nonextractable radiocarbon in house fly excreta was polar products not recovered from the water phase with ethyl acetate, but low levels of radiocarbon were not extracted from the paper itself.

Table I. Radiocarbon Recovery and Distribution 4 Days after Topical Treatment of Stable Flies and House Flies with [^{14}C]Diflubenzuron[a]

	% of applied radiocarbon			
	Stable fly		House fly	
Fraction	Treated male group	Treated female group	Treated male group	Treated female group
Flies, male				
Surface	43.3	1.3	38.6	1.0
Internal	6.3	2.3	0.4	0.4
Unextractable	0.2	0.1	0.1	0.1
Flies, female				
Surface	0.7	33.2	0.8	18.2
Internal	2.4	6.9	0.3	0.8
Unextractable	0.1	0.2	0.1	0.2
Excreta				
Extractable	34.8	37.4	46.6	45.8
Unextractable	0.5	0.6	5.0	5.8
Eggs				
Surface	0.1	0.1	<0.1	<0.1
Internal	0.4	0.8	<0.1	0.1
Unextractable	<0.1	<0.1	<0.1	<0.1
Total Recovery	88.9	83.0	91.9	72.4

[a] Ten flies of one sex were treated on the ventral abdominal surface with 2 μg of [^{14}C]diflubenzuron in 1 μL of acetone. The treated flies were held with ten flies of the opposite sex that were treated with acetone only.

Female stable flies treated with [^{14}C]diflubenzuron secreted almost 1% of the dose into the eggs, and untreated females caged with treated males suprisingly secreted 0.5% of the dose into the eggs (Table I). House flies treated with [^{14}C]diflubenzuron showed much less tendency toward secretion of radiocarbon into eggs. Only 0.1% of the dose appeared in eggs of treated female house flies, and <0.1% appeared in eggs of untreated females caged with treated males (Table I). Differences in numbers of eggs produced by the two fly species did not account for the almost tenfold higher total egg residues seen in stable flies, because average egg production by the treated females of each species was identical (84 eggs/female).

Untreated flies of one sex held with [^{14}C]diflubenzuron-treated flies of the opposite sex contained appreciable radiocarbon residues 4 days after treatment (Table I). About 3–4% of the applied dose was recovered in the untreated sexes of stable flies, whereas 1–1.5% of the dose applied to house flies was detected in the untreated sexes. The total recovery of the applied [^{14}C]diflubenzuron varied from 72–92%. We do not know why recoveries were lower from the treated female groups, particularly house flies, but the radiocarbon not accounted for may reflect experimental error because studies indicated that volatility was not likely a significant factor. We held 4 μg/cm^2 deposits of [^{14}C]diflubenzuron on glass surfaces in the laboratory for 7 days, with essentially no volatility loss.

Nature of Radiocarbon in Flies. Analysis of surface washes and internal extracts from stable flies and house flies exposed to [^{14}C]diflubenzuron revealed that these two species differ considerably in their capacity to metabolize

Table II. Metabolites in Stable Flies and House Flies 4 Days after Topical Treatment with [^{14}C]Diflubenzuron[a]

	Radiocarbon as indicated metabolite, % of sample[b]													
	Diflubenzuron		2,6-Difluorobenzamide		4-Chlorophenylurea		4-Chloroacetanilide		Unknown 2		Unknown 5		Unknown 6	
Fraction	SF[c]	HF[c]	SF	HF	SF	HF	SF	HF	SF	HF	SF	HF	SF	HF
Surface wash														
Treated male	99.4	97.4	0	0	0	0	0	0	0.6	2.6	0	0	0	0
Treated female	99.8	93.9	0	0	0	0	0	0	0.2	6.1	0	0	0	0
Untreated male	100	98.5	0	0	0	0	0	0	0	1.5	0	0	0	0
Untreated female	98.2	96.1	0	0	0	0	0	0	1.8	3.9	0	0	0	0
Internal extract														
Treated male	98.4	94.0	0.7	0	0.8	1.1	Tr	0	0	2.0	0	2.9	0	0
Treated female	99.1	89.6	0.4	0	0.5	1.4	Tr	0	0	1.0	0	6.0	0	2.0
Untreated male	98.8	96.6	0.7	0	0.5	0	0	0	0	0.7	0	2.7	0	0
Untreated female	99.1	93.4	0.3	0	0.6	1.3	0	0	0	1.6	0	3.7	0	0

[a] Ten flies of one sex were treated on the ventral abdominal surface with 2 μg of [^{14}C]diflubenzuron in 1 μL of acetone. The treated flies were held with ten flies of the opposite sex that were treated with acetone only. [b] Radioactive components comprising < 0.2% of the sample radiocarbon are recorded as Tr (trace). [c] SF (stable fly) and HF (house fly).

Table III. Metabolites in the Excreta of Stable Flies and House Flies 4 Days after Topical Treatment with [^{14}C]Diflubenzuron[a]

	% as indicated metabolite[b]			
	Stable fly		House fly	
Metabolite	Treated male	Treated female	Treated male	Treated female
Diflubenzuron	97.5	98.4	92.6	92.6
2,6-Difluorobenzamide	0.8	0.5	0.3	0.3
4-Chlorophenylurea	0.5	0.4	0.3	0.3
4-Chloroacetanilide	Tr	Tr	0	0
2,6-Difluorobenzoic acid	0.5	0.4	0	0
Unknown 1	0.2	Tr	0	Tr
Unknown 2	0.3	0.3	1.9	1.7
Unknown 3	0.2	Tr	0	0
Unknown 4	Tr	Tr	0.2	0.7
Unknown 5	Tr	Tr	4.3	4.0
Unknown 6	Tr	Tr	0.4	0.4

[a] Ten flies of one sex were treated on the ventral abdominal surface with 2 μg of [^{14}C]diflubenzuron in 1 μL of acetone. The treated flies were held with ten flies of the opposite sex that were treated with acetone only. [b] Radioactive components comprising < 0.2% of the sample radiocarbon are recorded as Tr (trace).

this insecticide (Table II). In stable flies, >98% of the radiocarbon in and on the flies was unmetabolized diflubenzuron, but in house flies, only 90–98% of the radiocarbon was recovered as the parent compound, depending upon the sample (Table II). Although the surface washes of both stable flies and house flies contained the same single metabolite (unknown 2, Table II), the nature and relative abundance of metabolites in the internal extracts differed considerably between the two species. Unknown 5, the major metabolite in extracts of house flies (Table II, Figure 1), was not seen in stable flies; and two of the stable fly metabolites, 2,6-difluorobenzamide and trace amounts of 4-chloroacetanilide, were not seen in extracts of house flies. 4-Chlorophenylurea was detected in extracts of flies of both species (Table II, Figure 1).

Studies with flies that were treated with [^{14}C]diflubenzuron and then analyzed immediately established that diflubenzuron does not undergo appreciable degradation during the extraction and work-up procedure itself. Only two radioactive components were detected in the extracts of these flies, unmetabolized diflubenzuron (99.8%) and a compound with chromatographic behavior identical with that of unknown 2 (0.2%).

Metabolites in the Excreta. In the extracts of excreta from [^{14}C]diflubenzuron-treated stable flies, 97–98% of the radiocarbon was unmetabolized diflubenzuron, but in the excreta of house flies, only about 93% of the extracted radiocarbon was the parent compound (Table III). Unknown 5 was the major metabolite in house fly excreta, but only trace amounts of this product were seen in stable fly excreta. The excreta contained the same identified metabolites as seen in the fly extracts, but the excreta from stable flies also contained small amounts of 2,6-difluorobenzoic acid (Table III).

Radiocarbon residues from acetone washes of the plastic holding cages of stable flies consisted primarily of unmetabolized diflubenzuron (>97%), and the only other radioactive component detected was unknown 2. Radiocarbon from the house fly cages also consisted of mainly diflubenzuron (>97%), but the other radioactive components included unknown 2 (1–2%) and trace amounts of unknowns 5 and 6. Combustion analysis of the feeding and watering pads showed only negligible radiocarbon residues.

Nature of Radiocarbon in Eggs. The eggs of both stable flies and house flies contained sufficient radiocarbon residues to permit analysis by TLC, although the very low levels of radiocarbon in house fly eggs necessitated extraction of a much larger sample. A single radioactive component was detected in the extracts from stable fly and house fly eggs, both from treated females and from untreated females held with treated males. Cochromatography studies with authentic diflubenzuron confirmed that the product was the unmetabolized parent compound.

Hatchability of Eggs from [^{14}C]Diflubenzuron-Treated Flies. Topical treatment of female stable flies with 2 μg of [^{14}C]diflubenzuron resulted in almost complete inhibition of hatch of eggs produced by the treated insects,

Table IV. Hatchability and Survival to the Adult Stage of Eggs from Stable Flies and House Flies Treated Topically with [^{14}C]Diflubenzuron[a]

Treatment group	Diflubenzuron in eggs, ppm		% egg hatch		% adult development[b]	
	SF	HF	SF	HF	SF	HF
Control[c]			92.4	93.4	92.2	93.5
Treated male	2.0	0.2	67.7	80.8	82.4	12.0
Treated female	5.1	0.5	3.1	91.2	0	13.4

[a] Ten flies of one sex were treated on the ventral abdominal surface with 2 μg of [^{14}C]diflubenzuron in 1 μL of acetone. The treated flies were held with ten flies of the opposite sex that were treated with acetone only. [b] Percent of hatched eggs reaching the adult stage. [c] Both sexes treated with 1 μL of acetone only.

and of the few eggs that did hatch, none successfully reached the adult stage (Table IV). Untreated female stable flies held and mated with treated males produced eggs that experienced a 25–30% reduction in hatchability, but about 80% of the hatched eggs survived to the adult stage. The [^{14}C]diflubenzuron treatment of house flies had little effect on egg hatchability, but development of the hatched eggs into adult insects was severely inhibited (Table IV). Less than 14% of the hatched eggs from either house fly treatment group successfully developed into adults (Table IV).

DISCUSSION

The current studies show that adult stable flies and house flies are capable of metabolizing diflubenzuron, although only to a limited degree. Results from our work with these two Dipteran species contrasts with those of previous studies with other insects, which have shown that the boll weevil (Still and Leopold, 1975), the salt marsh caterpillar (Metcalf et al., 1975), and *Pieris brassicae* larvae (Verloop and Ferrell, 1977) are apparently unable to metabolize diflubenzuron. House flies metabolize the compound to a greater extent than stable flies and appear to excrete diflubenzuron residues from the body more efficiently than stable flies, as evidenced by a greater percentage of the dose appearing in the excreta and generally less remaining on and in the treated insects (Table I). These findings may be attributed in part to greater metabolism by house flies to more polar and thus more easily excreted products. This hypothesis is supported by the presence of much higher amounts of unextractable radioactivity in the excreta of house flies (Table I), which presumably represents polar metabolites.

Flies degraded diflubenzuron by cleavage between the carbonyl and amide bonds to give 4-chlorophenylurea, 2,6-diflurobenzamide, 2,6-diflurobenzoic acid, and presumably 4-chloroaniline which was subsequently acetylated to the acetanilide. The considerable number of uncharacterized metabolites observed indicates that diflubenzuron is degraded by flies through several additional, undefined pathways. Aryl hydroxylation is apparently not a significant pathway for diflubenzuron degradation in flies, because none of the hydroxylated diflubenzuron metabolites that occurred in ruminants (Ivie, 1978) were seen in the current study.

Although up to 50% of the [^{14}C]diflubenzuron applied to the insects was recovered in the excreta (Table I), this radiocarbon likely does not represent exclusively fecal excretion of absorbed diflubenzuron. Almost certainly, a part of the radioactivity recovered from the linings of the holding cages reflected simply rub off of the topically applied compound. We likewise do not know whether the radioactivity detected in the untreated flies was absorbed through contact transfer from the treated flies or whether the radioactivity was picked up by contact with the excreta. Some direct transfer from the treated to the untreated sexes seems likely because of the considerable contact that occurs between the sexes, particularly during breeding.

The use of acetone as a carrier for the topical applications may have resulted in the deposition of rather large diflubenzuron crystals on the insects, because it is known that diflubenzuron solutions in acetone form large crystals (>100 μm) upon evaporation of the solvent on inert surfaces (Booth, 1977). If large crystals were indeed produced on the treated flies, the probability of rub-off would likely be increased, and certainly a larger crystal size would be expected to reduce the rate and extent of absorption.

The secretion of unmetabolized diflubenzuron into the eggs of adult flies treated topically with the insecticide is consistent with earlier reports that embryotoxicity seems to be involved in the ovicidal action of diflubenzuron in stable flies (Wright and Harris, 1976; Wright and Spates, 1976) and house flies (Grosscurt, 1976). Although earlier authors have used the term "sterility" to describe egg mortality caused by diflubenzuron, Grosscurt (1976) has pointed out that this activity is ovicidal and that diflubenzuron cannot be considered a chemosterilant by currently accepted definitions (Labrecque, 1968).

It was somewhat surprising that the untreated female stable flies held with treated males secreted about half as much diflubenzuron into the eggs as did treated females. However, this is consistent with our finding that the untreated female flies contained almost half as much internal radiocarbon residue at sacrifice as the treated females (Table I). House flies showed much less tendency toward secretion of diflubenzuron into the eggs than did stable flies, even though the experimental procedures were as identical as possible. Because house flies metabolized diflubenzuron to a greater extent than did stable flies and appeared to be more efficient in excreting the compound, the lower egg residues may have been partly a result of more rapid excretion by house flies and thus less diflubenzuron was available for secretion into the eggs.

House fly eggs contained about tenfold less diflubenzuron than eggs from comparably treated stable flies, and little effects were observed on house fly egg hatchability (Table IV). However, the low levels of diflubenzuron present in the eggs apparently exerted toxic effects on the developing larvae because only about 10–15% of the hatched larvae successfully reached the adult stage (Table IV). Thus, even though house flies show much less tendency toward secreting diflubenzuron into the eggs than do stable flies, the developing house fly larvae appear to be considerably more susceptible to the toxic effects of the egg-transferred insecticide. The effects on egg hatch and larval survival observed in these studies clearly result from absorption of diflubenzuron by the female and subsequent transfer to the eggs. This conclusion is based on the finding that little of the egg radiocarbon can be considered as surface residue (Table I), and on the previous observation that diflubenzuron shows no contact activity against house fly eggs (Grosscurt, 1976).

The effects reported here on egg hatchability in house flies treated with diflubenzuron contrasts somewhat with the results of Grosscurt (1976), who studied under very similar conditions the effects of diflubenzuron on egg hatchability in a different insecticide-susceptible house fly strain. Egg hatch in Grosscurt's study was severely inhibited by treatment of adult females with 1 μg topical

diflubenzuron (in acetone), whereas in our work house fly egg hatchability per se was not seriously affected by 2 μg of topical diflubenzuron. Variation in the amount of unmetabolized diflubenzuron secreted into the eggs of the two house fly strains was probably the immediate cause of the observed differences in egg hatch. It may be that detoxication mechanisms were more active in the house flies used in our studies, thus leading to less availability for secretion into eggs.

Our studies strongly indicate that the ovicidal effects observed in adult insects exposed to diflubenzuron by surface contact or orally (Moore and Taft, 1975; Grosscurt, 1976; Taft and Hopkins, 1975; Wright and Harris, 1976; Wright and Spates, 1976) is due to secretion of unmetabolized diflubenzuron into the eggs, where it exerts its toxic effects upon the developing embryo or larva. The indication in our studies that treated male insects can transfer appreciable quantities of diflubenzuron to untreated females provides a logical explanation for previous reports of intersex transfer of "sterility" among topically diflubenzuron treated biting flies (Wright and Harris, 1976; Wright and Spates, 1976) and boll weevils (Moore and Taft, 1975). The transfer of diflubenzuron residues from topically treated male to untreated female flies is almost certainly due to surface contact between the sexes and not through seminal fluid. Grosscurt (1976) found that injection of male house flies with 5 μg of diflubenzuron on the day before mating with untreated females had no effects on egg hatchability.

Insecticide treatment of adult insects to effect secretion into the eggs and subsequent toxicity to the developing embryos has previously been reported as a potentially useful application of certain compounds (Masner et al., 1970). Diflubenzuron appears to have considerable promise for such use, and its relatively high degree of metabolic stability in insects seems desirable from the standpoint of minimizing the amounts required for effective control.

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Chemical and Toxicologic Evaluation of Firemaster BP-6

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The flame retardant (Firemaster BP-6) involved in an accidental contamination of animal feed in Michigan has been subjected to chemical and toxicological analyses to establish whether brominated dibenzo-*p*-dioxin or dibenzofuran impurities were present. Firemaster BP-6 was found to contain at least 13 different bromobiphenyls and to be contaminated with approximately 200 ppm bromonaphthalenes. A polar fraction, which should contain the majority of any possible bromodibenzofuran and bromodibenzo-*p*-dioxin contaminants, was prepared. Although the polar fraction contained a large number of components, no bromodibenzofurans or bromodibenzo-*p*-dioxins were found. In addition, the polar fraction was relatively inert toxicologically compared to the nonpolar fraction or the unfractionated material. It is concluded that the observed biological effects are most probably due to bromobiphenyls.

In 1973, the accidental contamination of animal feeds with a fire retardant (Firemaster BP-6) comprised of a mixture of polybrominated biphenyls (PBBs) resulted in serious animal toxicosis and loss of production (Jackson and Halbert, 1974). Secondary contamination became so widespread that by 1975 over 30 000 livestock, 1 600 000 poultry, and thousands of pounds of their products were destroyed to limit human exposure (Dunckel, 1975). The effects of polychlorinated biphenyls (PCBs) are complicated by the presence of low levels of the very toxic chlorodibenzofurans (Kuratsune et al., 1976a,b). Therefore, we examined the Firemaster BP-6 for the presence of similar contaminants. Moreover, O'Keefe

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