Bull. Environm. Contam. Toxicol. 24, 921-928 (1980)

Organophosphate Risk Assessment: Field Testing of DEF with the Scaleless Chicken

B. W. Wilson[1], C. M. Cisson[1], W. R. Randall[1], J. E. Woodrow[2], J. N. Seiber[2], and J. B. Knaak[3]

Departments of [1]Avian Sciences and [2]Environmental Toxicology, University of California, Davis, 95616 and [3]Department of Food and Agriculture, Sacramento, CA 95814

Defoliants such as DEF (S,S,S- tributylphosphorotrithioate) are used to facilitate mechanical harvesting of cotton in California and Arizona. DEF is applied in the fall (October-November) when air movements are frequently restricted by inversions.

There is increasing concern regarding the safe use of this chemical, because it produces a delayed neurotoxicity in hens (BARON & JOHNSON 1964, ABOU-DONIA et al. 1980). DEF appears in the March 9, 1979 list of pre-RPAR reviewed chemicals, although there is presently no documentation of acute or delayed neurotoxicity in humans when it and related chemicals are used according to recommendations.

Toxicity tests involving experimental animals are carried out in the laboratory, limiting their direct application to the field. Most have fur or feathers that might interfere with dermal absorption of the compounds. One possible test animal is the "scaleless chicken" that has a defect in development, rendering it virtually devoid of feathers (ABBOTT & ASMUNDSON 1957). To find out whether the delayed neurotoxicity produced by DEF would occur under conditions used in the field, scaleless hens were put into cotton fields, exposing them to DEF during its application and observing them for signs of ataxia and other injury.

METHODS

Scaleless hens were bred and raised in the Department of Avian Sciences, UCD. Twenty-seven two-year-old hens were transported to Visalia, CA during the week of October 1, 1979, and housed in cages overnight. Food and water were provided ad lib.

The acute trial began on October 2, 1979. Repeated exposures of some birds were continued over the next five days. Two mature cotton fields (approximately 40 acres (16 ha) each, Diversified Farming Inc.) were treated with DEF-6 (0.72 kg/l, 6 lb/gal; Mobay Chemical Co.) at 0.37 gal/acre and Accelerate (amine salt of endothall (7-oxabicyclo-(2.2.1) heptane-2,3-dicarboxylic acid), 0.06 kg/l, 0.5 lbs/gal) at 0.19 gal/acre in 25 gal water/acre using a ground vehicle that sprayed

0007-4861/80/0024-0921 $01.60

8 rows simultaneously.

Each group of experimental animals consisted of 3 hens. In the acute trial, one control group of birds was approximately 400 m away from the site (OFF), and another was located in a dirt road between the two fields (ON). Two groups were placed within the rows of cotton (ROW) approximately 4 meters from the edge of the field, and sprayed directly by the rig. One set was placed in unsprayed cotton, 8 rows away from the path of the applicator (ADJ). Another set rode in a specially designed cage on the spray rig, immediately behind and slightly above the operator (RIG). Birds were provided with water and shade. Duplicate sets of mylar sheets (1858 cm^2) taped to pressed hardboard were placed at the OFF and ON locations. A small mylar sheet (464 cm^2) was placed on the spray rig next to the birds (RIG). The mylar sheets were left in place for the 7 hour duration of the experiment. Duplicate sets of petri dishes (67.5 cm^2) glued to pressed hardboard were placed at the ROW and ADJ locations. As soon as the spray rig passed their contents were rinsed into screw-cap bottles with acetone. The mylar sheets were folded and stored in wide mouth glass jars, and extracted with acetone prior to analysis. All samples were shipped to the laboratory in dry ice and stored at $-10^{o}C$ until analyzed. In addition, high and low volume air collectors were set up at the OFF, ON and RIG locations.

The repeated exposure groups consisted of one set placed directly in the path of the sprayer once a day (ROW), and another adjacent to it (ADJ) as described above. A control set (CON) was driven to the fields and returned to headquarters without leaving the automobile.

Birds were weighed and blood samples taken the day before they left Davis for Visalia. The birds on the acute test were returned to Davis on the same day; those that received repeated exposures were returned one week later, two days after their last exposure in the field. In both cases, blood samples were taken the day after their arrival in Davis. The birds were weighed and blood samples taken 2, 8, 15, 22 and 29 days after their first exposure and observed daily for signs of ataxia and other evidence of neuropathy or illness.

In order to determine if scaleless hens developed delayed neurotoxicity in a manner similar to that of normally feathered hens, a group of 3 birds was pretreated with atropine sulfate (20 mg/kg s.c.) and then injected with DEF (800 mg/kg s.c. technical grade, 94% purity, Mobay Chemical Co.) 30 minutes later. These birds received a second injection of atropine sulfate (20 mg/kg s.c.) 2 hours after treatment with DEF. Control hens received two injections of atropine sulfate as above. The birds were observed for changes in behavior daily. They were weighed and blood samples taken 2, 6, 13, and 20 days after treatment.

Plasma CHE activity was determined by a radiometric assay

(JOHNSON & RUSSELL 1975) using acetylcholine as substrate and BW 284c51 as a selective inhibitor of acetylcholinesterase. Plasma creatine kinase (CK) activity was determined by a spectrophotometric method (HESS et al. 1968).

DEF was analyzed by gas chromatography (WOODROW & SEIBER 1978; HERMANN & SEIBER 1979). Accelerate was also analyzed with a gas chromatograph using a flame ionization detector, measuring the response of the detector to the anhydride. An aliquot of the tank mix was diluted 1/10000 for DEF analysis and 1/2 for Accelerate. Minimum detectability was <0.2 ng DEF and <0.1 ug Accelerate.

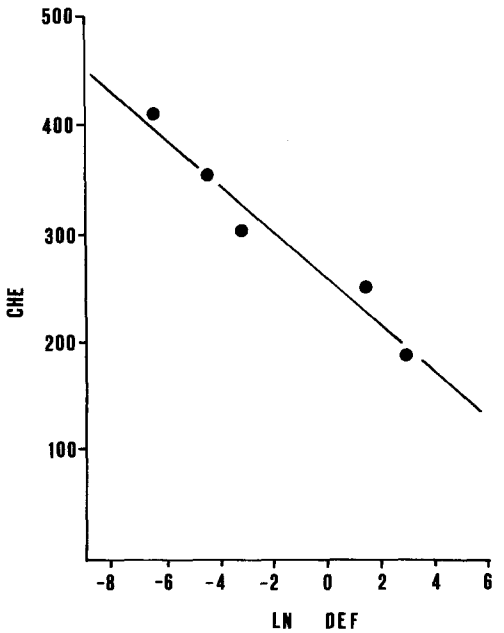

FIGURE 1: Plasma CHE in scaleless chickens after field applications of DEF. CHE in nmoles/min/ml; DEF in ln ug/cm^2. Single day exposures of groups in Table 1, omitting birds on the sprayer (RIG).

RESULTS

Levels of DEF to which the birds were exposed in the field ranged from negligible to a total of more than 100 ug/cm^2 (Table 1). CHE levels tended to decrease with increasing single exposures of DEF [with the exception of the birds riding on the rig (RIG)] falling to approximately 50% the CHE level of off-site birds. In addition, both ROW and ADJ birds showed decreased CHE levels with repeated exposures. CHE activity recovered in 2-3 weeks (Table 2).

TABLE 1
DEF, Plasma Protein and CHE

Group Site	DEF ug/cm2	Protein mg/ml	CHE u/ml	CHE Ratio
		Single Exposure		
OFF	0.0017	75.7	413	1
ADJ	0.0092	66.0	360	0.87
ON	0.04	72.3	300	0.73
ROW	4.4	57.3	251	0.61
ROW	17.7	69.8	190*	0.46
RIG	47.8	74.3	299	0.72
		Repeated Exposure		
ROW	108	75.6	223*	0.61
ADJ	3.9	72.8	323*	0.88
CON	-	76.2	368	1

DEF; means of 2-4 samples from either petri dishes exposed once to the applicator (ADJ,ROW) or mylar sheets exposed for ca. 7 hours. Protein and CHE value; means from 3 birds sampled 24 hours (acute) or 48 hours (repeated) after treatment. CHE in nmoles ACh hydrolzed /min/ml. of ACh hydrolyzed CHE Ratio; experimentals/appropriate control group (OFF or CON). Groups described in text. * Statistically different from day 0, $P<0.05$, t-test paired variates.

TABLE 2
Plasma CHE Levels

Group Site	Days After Exposure 0	2	8	15	22
		Single Exposure			
OFF	379	413	389	294	361
ADJ	380	360	385	300	333
ON	292	300	314	294	329
ROW	354	251	374	291	319
ROW	301	190*	252	290	309
RIG	379	299	332	320	408
		Repeated Exposure			
ROW	429	—	223*	323	352
ADJ	388	—	323*	349	337
CON	401	—	368	294	384
Grand Mean	367 ±45	NA	NA	306 ±20	348 ±32

CHE levels means of blood samples from 3 birds/ group. Values are in nmoles ACh hydrolzed /min/ml. Grand Means ± S.D. Standard deviations of groups and values for day 29 omitted for brevity. *Statistically different from day 0, $P<0.05$, t-test paired variates calculated for days 2 and 8.

Figure 1 shows there was a log-linear dose-response relationship for DEF and CHE, particularly for birds in the acute, one day study. Log-linear regression was CHE=260-21.4 ln (DEF); r=0.97; F-test: 51.61 (1,3), P<0.005. (r=0.81 and F-test significance was P<0.05 when all groups were included.)

The birds were observed for 30 days after they returned to Davis. None exhibited ataxia or other behavioral signs of delayed neurotoxicity. One died of unrelated causes.

Plasma CK activities rose (in one set by an average of more than 17-fold) but the increases did not correlate with the exposures of the birds to DEF. CK returned to normal within approximately 2 weeks.

Scaleless hens injected with DEF (800 mg/kg s.c.) in the laboratory developed ataxia after 12 days. Figure 2 shows the plasma CHE and CK activities found in these hens and the atropine-injected controls. CHE decreased to low levels as expected, in both scaleless (shown here) and normal birds (not shown), and, in this study, did not return to normal levels by 20 days. Interestingly, CK levels rose markedly after treatment with DEF, but did not rise in the controls. Plasma CK activity also rose in birds treated with DFP and parathion (Cisson et al., in preparation).

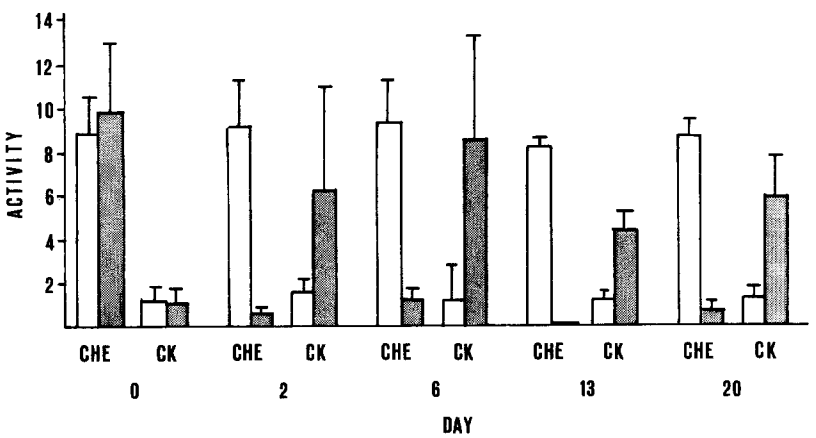

FIGURE 2: Plasma levels of CHE and CK in scaleless chickens given 800 mg/kg DEF ± 40 mg/kg atropine or atropine alone and sampled on the days indicated. 3 birds per group. Dark bars are for DEF treated birds.

The DEF levels to which the applicator was exposed during

the study are shown in Table 3. Samples were from 4 x 4 cm gauze patches inside and outside a coverall worn by the applicator for the 7 hour duration of the field test. Values outside the coveralls were as high as 250-260 ug/cm^2, and ranged from above 1 to almost 7 ug/cm^2 inside of the garment, comparable to levels to which the chickens were exposed.

TABLE 3
Exposure of Applicator

Coverall Region	ug/cm^2 Sleeve	Shoulder	Chest	Thigh	Leg
Outside	260	24.3	23.9	102	44.0
	254	50.7	68.4	84.8	49.5
	68.4		47.5	58.9	17.5
Inside		1.37	2.66	6.76	
		1.66	2.84	4.55	
			3.13	6.44	

DEF levels measured in the air and on the surfaces of mylar and petri dishes during the 7 hour interval or the experiment are compared in Table 4. Although similar trends were exhibited by both kinds of measurements, the relatively lower levels in the air samples, and calculations of exposure based on an approximate respiration rate of 1 l/min for the birds suggest that the major route of exposure was dermal rather than by inhalation.

TABLE 4
Fallout and Air Levels of DEF

Group Site	Fallout ug/cm^2	Air Samples DEF ug	Volume m3	Conc ug/m^3
OFF	0.0017	45.2	409	0.111
ON	0.040	2900	470	6.2
RIG	47.8	300	21.8	13.8

The tank mix contained approximately 10.4 mg/ml of DEF and 0.5 mg/ml of Accelerate, similar to the ratios found in the samples collected. (In addition, there was a good correlation between the relatively high values of DEF for the "On-site" controls (ON) in Table 1 and the fact that formulation was accidently spilled from the tanker onto the dirt road within 15 m of the birds).

DISCUSSION

The results establish the feasibility of field studies of chickens without feathers. The ability of the birds to survive without noticeable discomfort under field conditions permits chronic studies that approximate the exposures obtained by

humans, both during application of the defoliant and during harvesting of the crop.

The regularity of the dose-response relationship between DEF fallout and plasma CHE levels extending over 5 orders of magnitude is almost as "well-behaved" under field conditions as one might expect in the laboratory. The lack of a large decrease of CHE in the birds that rode on the applicator may reflect a problem in design. The cage was lined with thick rubberized screening to prevent any injury to the birds that may have occured while being jostled by the rig and a nylon tarp was suspended 0.3-0.5 m above the cage to shade the birds. These measures may have impeded fallout of DEF in the cage, reducing levels below those found on the mylar adjacent to it.

The laboratory studies establish that the scaleless chicken develops delayed neurotoxicity. Its hardiness in the field and its lack of feathers suggest it will be a useful animal model for studies of dermally applied toxicants. Other features that favor absorption include hypokeratinization and increased capillarity of its skin (SAWYER et al. 1972).

The mechanism of the delayed neurotoxicity is not known (JOHNSON 1975). "Early warning" tests for it are lacking. Some work suggests there is initial damage at the periphery, perhaps involving focal lesions in axons (BOULDIN & CAVANAGH 1979) and the neuromuscular junction (GLAZER et al. 1978), which could be detected by biochemical and electrophysiological tests used clinically to study neuromuscular disorders, such as the CK test applied here. The laboratory data suggest that serum enzymes like CK may be useful markers for organophosphate exposure. However the conditions of this test must be controlled. The increase of CK in all birds taken to Visalia, regardless of their exposure to DEF in the field suggests that the rigor of the trip may have stressed the birds and increased CK levels. Plasma CK activity in humans is known to increase under stress, such as after heavy exercise (FOWLER et al. 1968).

The experiments bring an experimental animal, the scaleless chicken, into the field to establish safe levels for use of organophosphates, particularly those causing delayed neurotoxicity. The use of such "sentinal" birds may be a model for future ways to bridge the gap between laboratory findings and their application.

ACKNOWLEDGEMENTS

Supported in part by grants from Western Regional Pesticide Impact Assessment Program and NIH ES 00202. We thank Assistant Agricultural Commissioner W. R. Clark and Deputy Commissioners L. Thomas, and T. Griffiths for their assistance.

REFERENCES

ABBOTT, U.K. and V.S. ASMUNDSON: J. Hered. 48:63 (1957).

ABOU-DONIA, M.B., D.G. GRAHAM, P.R. TIMMONS, and B. L. REICHERT: Neurotoxicology, In Press (1980).

BARON, R.L. and H. JOHNSON: Brit. J. Pharmacol. 23:295 (1964).

BOULDIN, T.W. and J.B. CAVANAGH: Am. J. Pathol 94: 241 (1979).

FOWLER, W.M., Jr., G.W. GARDNER, H.H. KAZERUNIAN and W.A. LAUVSTAD. Arch. Phys. Med. and Rehab., 49:554 (1968).

GLAZER, E.J., T. BAKER, and W.F. RIKER, Jr., J. Neurocytol. 7:741 (1978).

HERMANN, B.W. and J.N. SEIBER: ACS Meeting, (1979).

HESS, J.W., K.J. MURDOCK, and G.J.W. NATHO: Am. J. Clin. Pathol. 50:89 (1968).

JOHNSON, C.D. and R.L. RUSSELL: Anal Biochem. 64:229 (1975).

JOHNSON, M.K: CRC Crit. Rev. Toxicol. 3:289 (1975).

SAWYER, R.H., U.K. ABBOTT, and J.D. TRELFORD: Science 175:527 (1972).

WOODROW, J.E. and J.N. SEIBER: Anal. Chem. 50:1229 (1978).