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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

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SUBJECT: Association of Aerial Pesticide Spraying with Reye Syndrome.

Background:

Aerial spraying of pesticides has been implicated as a possible factor in the etiology of Reye Syndrome. Reye Syndrome occurs in children usually following a viral infection, commonly influenza B, A (H1N1) or varicella. It is characterized by an acute non-inflammatory encephalopathy with (1) microvesicular fatty metaphorphosis of the liver or (2) a serum glutamic oxaloacetic transaminase, a serum glutamic pyruvic transaminase or serum ammonia greater than 3 times normal levels (Centers for Disease Control, 1980, 29:321). The fatality rate has declined from 42% in 1976-1977 to 28% in 1980-1981 (Centers for Disease Control, 1982, 31:53).

Epidemiology

There is only one epidemiology study associating aerial pesticide spraying with Reye Syndrome-first mentioned in Lancet (Crocker, J. D. S., et al, 1974) and later mentioned in a similar report in Science (Crocker, J. F. S., et al., 1976). No details were given, they merely mentioned that some children with Reye Syndrome lived in the vicinity of forest spraying operations. The full report finally appeared in Chemosphere (Bagnell, P. C., J. F. S. Crocker, and R. O. Ozere, 1978). Fourteen of seventeen patients came from areas of New Brunswick whereas the remaining three (including the only urban dweller) came from Nova Scotia and Prince Edward's Island. The authors state that New Brunswick is "the only area (in Eastern Canada) where amounts of a chemical are used in the environment in such concentrated and consistent quantities on a yearly basis".

There are many problems with this study. Only seventeen cases were studied and actual exposure to aerial spraying was not demonstrated. The cases "tended to cluster in the winter -spring months" (the statistics were not given). This would rule out immediate exposure to the predominantly summer spraying. Much larger epidemiological surveys in the United States have confirmed the late winter clustering of cases associated with the influenza season (Hattwick, M. and R. Sagetta, 1978) (Nelson, D. B., et al., 1978) (Centers for Disease Control, Annual Summary 1980). The Bagnell, Crocker, and Ozere report presents the high rural/urban ratio of their seventeen cases as further support for the association of Reye Syndrome with aerial spraying. An Ohio study (Nelson, D. B., et al., 1978) on 190 retrospective case from 1973 to 1977 stated that "previously published observations that Reye Syndrome occurs primarily in suburban - rural areas, and rarely occurs in the iner city, are not supported" (by the Ohio study). The Bagnell, Crocker, and Ozere report also mentions (but does not support with data) that "the two common factors in most of our patient population were exposure to salicylates and geographic clustering contiguous to aerial spraying of forest land". The association of Reye Syndrome with salicylate usage has been further studied (Centers for Disease Control, 1980, 29: 532). The Centers for Disease Control (CDC) concluded that the evidence for the association between Reye Syndrome and salicylate usage was sufficient (although not proven) to advise "physicians and parents of the possible increased risk of Reye Syndrome associated with the use of salicylates for children with chickenpox or influenza-like illness" (Centers for Disease Control, 1982, 31: 53)

Experimental Models

The groups at Dalhousie University, Nova Scotia, that had noticed the possible association of Reye Syndrome with pesticide spraying developed a mouse model system.

Swiss white mice were painted on the abdomens or backs once daily from 24 hours after birth to day 11 with solutions of pesticides, emulsifiers in a corn oil base and solvent, and corn oil controls. A sublethal dose of mouse encephalomyocarditis virus was injected subcutaneously at day 13. "Most deaths" occurred within 5 days. The survivors were necropsied at day 10 or day 23 (not clearly specified) and brain and liver sections were taken for histological examination.

It is difficult to fully evaluated this study since parts of it were reported at different times with conflicting details. The initial publication (Crocker, J. F. S., et al. 1974) reported testing a corn oil control, approximately 124 mg/kg DDT in corn oil, commercial formulated fenitrothion, and DDT + formulated fenitrothion.

Test Material	Number of Mice Tested	Approximate % Survivors Virus Titre 0	$10^{-8.5}$	10^{-9}	$10^{-9.5}$
Corn Oil	Not reported	"All Survived"			
DDT	130	92	82	92	92
Fenitrothion	135	96	92	90	90
DDT + Fenitrothion	247	92	64	32	33

These results were interpolated from the graphs presented in the report.

The second report (Crocker, J. F. S., et al., 1976) evidently republished the results on DDT, formulated Fenitrothion, and DDT + Fenitrothion. Corn oil controls were reported with results different from the first publication. Purified fenitrothrion and DDT (> 99% pure) and a solvent/emulsifier mixture (Aerotex 3470, Toximul MP8 and Atlox 3409, as found in formulated fenitrothion) were tested.

Test Material	Number of Mice Tested	Approximate % Survivors Virus Titre 0	$10^{-8.5}$	10^{-9}	$10^{-9.5}$
Corn Oil	42	87	68	?	?
DDT + 3.8% Fenitrothion	57	90	55	90	?
Solvent + Emulsifier	181	85	28	15	72

Histology of the brain sections reported "changes compatible with edema" but no gross morphological change by light microscopy. "There was no correlation between the severity of the histopathological changes and the increased mortality-rate". Fatty changes in the liver were also noted: a diffuse fine pattern was seen in animals treated with the solvents, emulsifiers, and virus; and large fat droplets were seen in the liver periportal areas after treatment with DDT and virus. There are some discrepancies in the reporting of these findings between the two papers and the frequencies, number of animals examined and other quantitative parameters are not given.

A similar experiment was performed with Hormel Pigmee piglets (Crocker, J. F. S., et al., 1978). Due to discrepancies between the text and the figures it was not possible to determine dosages or results exactly. For example: the text says that the controls were divided into two groups, (1) no chemical administration and virus injected intracerebrally and (2) corn oil "exposed" and virus given orally, but the only numbers given as results are in the figures which do not distinguish between the two groups. Sections were taken of the livers and brains and it was stated that the "pathological picture would appear to have no consistant pattern". The pigs were evidently treated by gavage with various combinations of fenitrothion, emulsifiers, solvents, DDT and corn oil.

Two assay systems were developed using mammalian cells in culture. A group at Dalhousie University (Rozee, K. R., et al., 1978) (Lee, S. H. S., 1978) exposed monolayers of mouse L-929 cells for 18 hours to a test chemical, washed, and inoculated with Vesicular stomatitis virus (VSV). After 90 minutes of absorption the monolayer was overlayed with growth medium containing 0.6% agarose to keep the virus from spreading and incubated 2 days. The number of infected cells was estimated by counting the plaques formed in the cultures. The ratio of the treated culture plaques to the control culture plaques was called the viral enhancement ratio. A ratio of 2 or greater was arbitrarily choosen as enhancing . Fenitrothion (FT) (95% pure), Aerotex 3470 (a fenitrothion solvent), and two aerial spraying emulsifiers: Toximul MP8 and Atlox 3409 were tested.

	Enhancement Ratio at Concentration (ppm)				
	0.10	0.25	1.0	2.5	10.0
FT	0.92	0.96	1.12	2.49	1.18
Aerotex	0.90	0.94	0.76	0.96	0.74
Toximul MP8	0.78	0.88	1.15	1.88	4.19
Atlox 3409	0.82	0.88	1.05	2.92	3.60

By their criteria, fenitrothion is slightly enhancing, and both Toximul MP8 and Atlox 3409 are stronger enhancing agents. In a later experiment, no synergistic effects were noted with a mixture of FT, Aerotex, and Toximul.

Toximul MP8 was used as the test material to examine the effects on other viruses. The infectivity of VSV and enaphalomyocarditis virus (single stranded RNA viruses) was enhanced while the double stranded viruses vaccinia, Herpesvirus hominis type 1 and reovirus type 2 were not enhanced.

Hela cells were found to be more sensitive to viral enhancement by Toximul MP8 than L-929. Seventeen emulsifiers were tested using Hela cells and VSV.

	Enhancement Ratio at Concentration of (ppm)						
Emulsifier	0.1	1	5	10	50	100	Enhancement
Toximul D	1.0	1.8	8.0	Toxic	-	-	+
Toximul R	1.3	1.6	7.1	Toxic	-	-	+
Toximul MP8	1.0	1.0	2.0	14.5	-	-	+
Toximul MP10	0.9	0.8	1.1	1.4	Toxic	Toxic	-
Pluronic L31	0.7	0.7	0.8	0.8	1.1	Toxic	-
Plurafac RA30	0.8	1.0	0.8	1.6	1.2	Toxic	-
Pluronic L64	0.5	11.0	Toxic	Toxic	-	-	+
Brij 56	1.6	9.9	Toxic	Toxic	-	-	+
Atlox 3409	0.8	1.1	1.9	3.6	-	-	+
Polytergent FL62	0.8	1.4	11.0	11.1	-	-	+
Richonate 40B	1.1	0.7	1.4	1.4	1.1	Toxic	-
Varine 17	0.7	1.4	1.3	1.7	Toxic	Toxic	-
Marlophen 810	0.9	12.7?	Toxic	Toxic	-	-	+
Sterox SL	1.0	0.8	-	Toxic	-	Toxic	-
Nonidet P-40	1.1	1.3	-	1.5	-	Toxic	-
Triton X-100	1.0	1.1	-	1.2	-	Toxic	-
Sodium dodecyl sulfate	-	0.8	-	2.1	-	Toxic	+

Another type of viral enhancement assay has been developed by M. Jerkofsky at the University of Maine (Abrahamsen, L. H. and M. Jerkofsky, 1981). She exposed monolayers of primary human embryonic lung cells (HEL) to the pesticide or inerts for 18 hours. The cells were then washed and incubated with wild-type (clinically isolated) varicella-zoster (VS) virus until plaques were visable (3 to 5 days). The cultures were then trypsinized and the number of viruses (viral infection centers) estimated by a standard viral titration method (inoculation of dilutions onto new monolayers for counting).

The compounds tested were: Sevin 4 oil (a carbaryl formulation), carbaryl (99.9% pure), and the base oil plus inert ingredients with carbaryl. The enhancement index was calculated as the ratio of number of infectious centers in the treated cells to the number of infectious centers in the controls.

Effect of Seven and Components on the Growth of VZ in HEL Cells

Compound	Infectious Centers per Culture: Treated	Control	Enhancement Index
Sevin (4 ppm)	3.4×10^6		9.18
Sevin (4 ppm)	3.8×10^6		10.2
Carbaryl (5 ppm)	5.0×10^6		13.5
Carbaryl (5 ppm)	3.9×10^6		10.5
Base Oil	3.3×10^5		0.89
Base Oil	3.4×10^5		0.91
Control		3.7×10^5	1

Effect of Decreasing Concentrations of Carbarly on Viral Enhancement

Carbaryl (ppm)	Infectious Centers per Culture: Treated	Control	Enhancement Index
18	9.7×10^5		2.0
3.7	8.0×10^5		1.7
0.7	7.8×10^5		1.6
0.37	4.7×10^5		1.0
0		4.7×10^5	1.0

Comparing these tables reveals that 5 ppm of Carbaryl produced an average enhancement index of 12.0 in one experiment whereas in the second experiment a concentration of 18 ppm produced an index of only 2.0. It was reported that enhancement indexes for carbaryl ranged from 2 to 50 in similar experiments so that variation is a problem with this particular assay. It has been found that different strains of viruses produce variable results: lab adapted strains do not respond as well as wild types (M. Jerkofsky, personal communication, 1982). This group is currently studying changes in cellular lipid metabolism induced in this system.

Discussion

The epidemiological studies are not sufficient to support an association of aerial pesticide spraying with Reye Syndrome and, in fact, the authors state "the results of these experiments are not sufficient to prove that Reye's Syndrome in man is caused by a chemical-virus interaction" (Bagnell, P. C., et al., 1978). More recent epidemiological studies have not shown a rural/urban bias and an entirely different factor, salicylate usage, is now implicated (Centers for Disease Control, 1982, 31: 53).

The mouse studies (Crocker, J. F. S., et al., 1974) were originally reviewed in Toxicology Branch (Budd, E., memo to Dr. Paynter, 1976) and found to be inadequate. The later publications of these studies (Crocker, J. F. S., et al., 1976) (Crocker, J. F. S., et al., 1978) added more information however the experiments are still not presented well enough to evaluate completely. Even if we assume the experiments to have been done in a valid manner, the results as reported would not be sufficient to associate pesticides with Reye Syndrome. The increase in mortality from the virus in the treated mice is not unexpected at the toxic levels of the compounds administered (from 8% mortality with DDT to 15% with inerts when no virus is administered). In fact, the corn oil control alone produced approximately 13% mortality by itself and 32% mortality after injection of the lowest dose of virus.

The report of increased fat distribution in the liver is of more concern since this is one of the findings in Reye Syndrome. Increased lipid deposition in the liver is not unusual, however, and is commonly seen as a response to many toxins. If the exposure to the emulsifier as tested here was responsible, in conjunction with a virus, for Reye Syndrome , it would be necessary for the affected person to be exposed to a high concentration of the emulsifier at the same time as the viral infection. The majority of Reye Syndrome cases appear in the late winter which makes a connection between aerial spraying and pesticide usage unlikely.

The pig study (Crocker, J. F. S., et al., 1978) produced no pathological evidence linking these compounds with Reye Syndrome and was not reported well enough to review.

The viral enhancement assay as performed at Dalhousie University (Rozee, K. E., et al., 1978) actually measures viral infectivity rather than viral replication. It was sufficiently well done and described to show that the chemicals studied, and in particular some of the emulsifiers, at near toxic levels, can increase the susceptability of these cells in culture to single stranded RNA viruses. It would be pure conjecture to associate these findings with Reye Syndrome especially since the epidemiology and animal studies reviewed here are so weak. It has not been shown in cases of Reye Syndrome tht the prodromal viral illness is more severe than that of control groups. One would suspect that many chemicals other than pesticide-associated chemicals would be able to produce viral enhancement, and, in fact, an assay quite similar in design to the Dalhousie University assay has been recommended as a method to screen carcinogens (Yoshikura, H. et al., 1979). In this assay, T. Matsushima chose an enhancement index of 5 to indicate definate enhancement as compared to an index of 2 in the Dalhousie University assay. He states "Whatever the mechanism of the enhancing effect is, this effect may be an essential action of carcinogens in general".

The second viral enhancement assay (Abrahamsen, L. H. and M. Jerkofsy, 1981) measure viral replication as opposed to viral infection. It is well enough performed and reported to show enhanced viral replication by carbaryl in HEL cells. It is interesting that the inerts were not enhancing since the Dalhousie studies showed the opposite effect.

As mentioned before the association of mammalian cells in culture such as this with Reye Syndrome would be pure conjecture without epidemiological evidence and more characteristic animal models.

Recommendations and Conclusions

1. Epidemiological studies do not show any connection between Reye Syndrome and aerial spraying.

2. The animal models are not adequate to show a connection between Reye Syndrome and pesticides or their inert ingredients.

3. The findings of the viral enhancement assays are probably not unique to pesticides and pesticide-associated inerts ingredients. Future assays should test other materials as well. A positive result in a viral enhancement assay can not be directly associated with Reye Syndrome. It is possible, however, that these chemicals could be mutagens, or otherwise genotoxic, and should be tested for this.

Toxicology Branch will continue to survey Reye Syndrome reports and associated animal and in vitro assays, and will reconsider its position if more substancial evidence is reported.

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cc: Caswell file, Fenitrothion #373
Caswell file, Matacil #360
Caswell file, Carbaryl #160
David Ritter, Reference inerts
Henry Spencer, Reference Forest uses

OPP:HED:TOX: W.SCHNEIDER:sb 4/22/82 X73710 Rm 816 #m12

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