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5. These are: *Aphis fabae, Rhopalosiphum padi, Schizaphis graminum, Hyadaphis erysimi, Macrosiphum euphorbiae, Acyrthosiphon pisum, A. solani,* and *Myzus persicae* (subfamily Aphidinae); and *Chaitophorus viminalis, C. populicola,* and *Sipha kurdjumovi* (subfamily Chaitophorinae).
6. Ant colonies were maintained in soil in metal bushel baskets in a laboratory at 22° ± 2°C and 15 hours of light per day. Cotton string run through plastic tubing was used to connect ant colonies with plants bearing aphid colonies. Aphid-ant interactions were observed the first 4 days after the introduction of ants.
7. Clusters of 3 to 30 aphids were exposed to cornicle droplets produced by gently squeezing an aphid of the same species. Each species was tested 12 to 25 times.
8. Ant-attended aphids were exposed to ants for 4 days during which aphids developed from first and second instar nymphs to fourth instar nymphs and young adults. Ants were removed 15 minutes to 1 hour prior to testing aphids. Clusters of 5 to 27 aphids were tested 18 times with cornicle droplets as reported above (*7*), or with *trans*-β-farnesene in a methanol solution. Paper triangles that hold approximately 1 μl of solution were held within 1 cm of aphids for 1 minute. Aphids did not respond to other aphids that did not produce cornicle droplets or to paper triangles treated with methanol alone.
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Lethal Interaction of Ubiquitous Insecticide Carriers with Virus

Abstract. *Large quantities of presumably nontoxic petroleum oil by-products are introduced into the environment as pesticide dispersal agents and emulsifiers. An increase in viral lethality with a concomitant influence on the liver and central nervous system occurs in young mice previously primed with such chemicals.*

Impurities or by-products which occur in pesticides and industrial compounds may cause disease in organisms that have been exposed to these agents. The toxicity or lethality (or both) associated with the herbicide 2,4,5-T (*1*), chlorinated phenols (*2*), polychlorinated biphenyls (*3*), and diphenylamine (*4*) are due to impurities introduced during manufacture or mixing of these compounds or to impurities formed during their storage or environmental breakdown. Among the pesticides known to have additives that enhance their toxicity are aldrin (*5*), chlordane *p,p'*-DDD, diazinon, and trichlorofon (*6*). Contamination of formulations by nonbiodegradable persistent material is a current and ongoing problem with an impact on animal and human health (*7*). For example, a group of Canadian children living within the area of a forest sprayed with fenitrothion and DDT suffered a particular combination of central nervous system and liver pathology after they had been exposed to a virus. We observed that when young mice were first exposed to certain combinations of the above-mentioned insecticides and then infected with a nonlethal dose of mouse encephalomyocarditis (EMC) virus, the effect of the virus was enhanced (*8*). The insecticide used in the forest spray area was applied as an emulsified concentrate consisting of toxicant, one or more solvents, and a blend of emulsifiers. The solvents were used as diluents and together with emulsifiers are useful in the even dispersal of the insecticide particles. Emulsifiers also act as wetting agents on foliage (*9*). The type of formulation in which a pesticide is dispersed can significantly affect the toxicity hazard (*10*).

Little work has been done on insecticide carriers, and toxicological data are almost nonexistent. These carriers are usually considered nontoxic and therefore find wide use in industrial and domestic products. In addition they have received very little attention from environmental research groups or agencies. Consequently, we examined the toxicity of the particular solvent carriers in the insecticides. Our study shows that the emulsifiers and solvents are, in fact, active in vivo chemicals which influence viral lethality.

Swiss white mice (Biobreeding, Ottawa) from an outbred strain (ICR) were mated in our own laboratories and allowed to deliver their young (884 mice). Contact of the newborn mice with the chemical under test was started at 24 hours of age. All groups of mice were studied simultaneously. To avoid inbreeding, we purchased new females for each group of experiments.

Purified fenitrothion and DDT were obtained from commercial sources and their purity was confirmed by gas chromatographic analysis (> 99 percent).

Fenitrothion was prepared in a solvent (Aerotex 3470, Texaco of Canada) with two emulsifiers (Toximul MP8, Chas. Tennant & Co. of Canada Ltd., Toronto, Ontario; Atlox 3409, Atlas Chemical Industry Ltd., Brantford, Ontario). Samples of each emulsifier and solvent (as used) were obtained from the suppliers. Thin-layer chromatographic analysis of the solvent and the emulsifiers gave several bands; subsequent examination of these fractions by gas chromatography indicated that several chemical components were present in each band. The ultraviolet absorption spectra of the solvent and emulsifiers showed maxima at 274, 265, and 255 nm; these are typical values exhibited by alkylated aromatic compounds (*11*). Corn oil was used as a control carrier because of its nontoxic properties and solubility characteristics.

Dosages of insecticides were based on established median lethal doses (LD_{50}) (*12*) and on our own trials in which the toxicity of various dosages was determined relative to the age of the animals (DDT, 124 mg per kilogram body weight and fenitrothion, 7.6 percent, by volume). The emulsifiers and solvent were mixed with corn oil (7 : 1000); this is the ratio for the carrier of fenitrothion used in this commercial spray formulation. Each solution was applied with a small camel-hair brush to the abdomens of the young animals. Solutions were painted once daily from 24 hours after birth (day 1) until day 11. The following solutions in corn oil base were applied: (i) corn oil alone; (ii) pure DDT; (iii) commercial fenitrothion (which contains the emulsifiers and the solvent); (iv) DDT plus the commercial fenitrothion; (v) DDT plus 3.8 percent pure fenitrothion (equivalent to DDT plus commercial fenitrothion without emulsifiers and solvent); and (vi) emulsifiers and solvent.

Stocks of EMC virus, originally obtained from the National Institute for Medical Research, Mill Hill, London, and supplied as the primary mouse-kidney cell yield, were used. The titer of the stock virus was between 10^6 and 10^7 median tissue culture infectious doses ($TCID_{50}$) per milliliter. The animals were observed for the development of symptoms.

Most deaths occurred within 5 days of viral injection. Long-term survivors were observed until day 23, and then were killed by cervical dislocation. Specimens of liver and brain were obtained, fixed in formalin, stained by hematoxylin and eosin, and observed by light microscopy. Other matched liver specimens were frozen and stained by oil-red-O to visualize localization of lipids. Additional tissue was obtained from the affected animals at the time of death for viral and

chemical studies. Death was generally preceded by convulsions in those animals that were injected with virus. The estimates of the probabilities of survival were calculated by the Kaplan and Meier (*13*) "product-limit" method. Then approximate standard errors and 95 percent confidence limits were determined for each total group at the time of the viral injections (day 13), and for each subgroup receiving the 10^{-9} dilution of stock virus 6 days later (day 19).

The mortality rate was much higher in animals exposed to the mixture of solvent and emulsifier and subsequently injected with EMC virus (Fig. 1F) than in those that were given corn oil alone (Fig. 1A) or pure insecticides (singly or in combination, Fig. 1, B and E). The results shown in Fig. 1C indicate that fenitrothion would appear to have a protective effect on this lethal interaction; this protective effect has been observed with structurally related organophosphates in other toxicity studies (*14*). The results shown in Fig. 1D would indicate that DDT may partially counteract this protective effect since the mortality rates shown in Fig. 1D are significantly higher than those shown in Fig. 1C. The small dose of virus required to produce death in these animals is below our LD_{50} for mice not primed by chemical toxin as shown by the control group (Fig. 1A).

In sections of the liver and brain stained with hematoxylin and eosin there were no signs of inflammatory reaction and no areas of gross cellular distortion or cell necrosis. The carrier group that had been exposed to virus had normal cellular alignment with numerous fatty vacuoles that did not prominently displace the nuclei. In frozen sections stained for lipids (oil-red-O), fine fat droplets were evenly dispersed throughout the liver lobule of the animals. In contrast, animals exposed to DDT and virus showed consistent large "fat lakes" distributed periportally.

Histological evaluation of the brain stained with hematoxylin and eosin showed no gross morphological change by regular light microscopy and no detectable inflammatory or necrotic defects; however, changes compatible with edema were present.

Lewin *et al.* (*15*) noted an increase in the mortality of mice when various solvents were combined with polychlorinated biphenyls and DDT and injected intraperitoneally. The variations reported (*16*) concerning the liver toxicity of several organophosphates could conceivably be explained as being due to carrier substances rather than the insecticides. We have shown that the emulsifiers and solvents in commercial insecticide spray projects can increase the lethality of viruses. The chemical "priming" of a host to respond unfavorably to virus infection on the basis of interaction could have various disease implications in man.

Fatty liver changes with a large droplet pattern have been noted in many situations, from starvation to diseases caused by toxins (*17*). There are two patterns of fat distribution in the liver of our experimental animals: a diffuse fine pattern seen in those animals treated with carrier and then injected with virus, and large fat droplets in the periportal areas in animals treated with DDT and virus. It is possible that the observed fine droplet pattern is due to the action of the emulsifier on fat within the liver cells in the animals given carrier and virus. The pattern of fine droplet liver cell staining has been stated to be characteristic of Reye's syndrome in children (*18*). This syndrome was the condition in the children which originally stimulated our concern in this area.

Reye's syndrome, a fatty hepatomegaly with nonspecific encephalopathy, has been reported in association with outbreaks of influenza B and infections with varicella zoster, coxsackie, echovirus, adenovirus, parainfluenza, and herpes simplex (*19*). However, a direct viral etiology has not been established (*20*). One of the theories concerning the etiology of Reye's syndrome postulates that environmental factors may be implicated (*19, 21*). Olson *et al.* (*22*) have previously theorized that Reye's syndrome could be a toxin-related disease, but from available studies it has been difficult to implicate a single toxic agent.

There are several possible explanations that could be offered for the observed interaction of virus and chemical noted in our experiments.

1) A virus-chemical toxin interaction. Influenza virus, one of the most frequently reported with human cases of

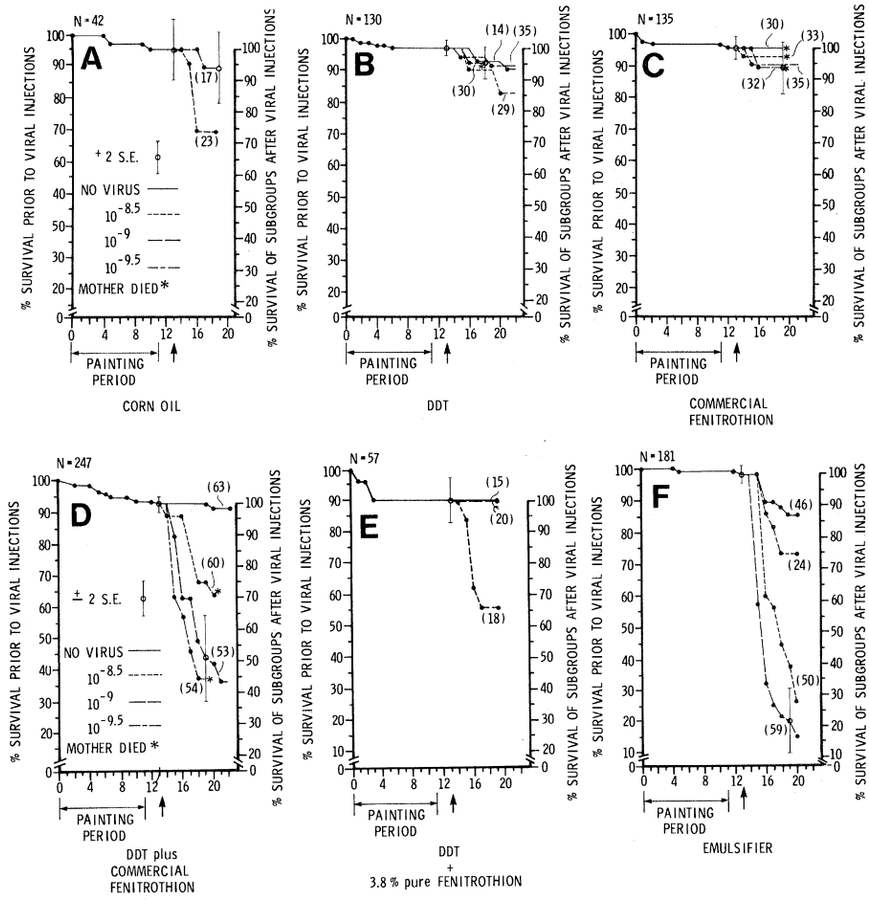

Fig. 1. Survival curves of newborn mice. Zero time on abscissa is 24 hours of age. Painting period is contact exposure to chemical and combinations as noted in graph key (A). The ordinate on the left side of each graph is the percentage of animals alive at 24 hours of age. The ordinate on the right side of each graph is the percentage of animals at the time of virus exposure. The median dose of virus dilution (10^{-9}) of a stock containing 10^6 to 10^7 TCIQ/ml was chosen for calculation of confidence limits. The maximum mortality (85 percent) occurred in animals exposed to virus who had contact with emulsifier (including solvent).

Reye's syndrome, is postulated to have toxic qualities (*23*). This may or may not be related to its neuramidinase-like activity. If other viruses—for example, EMC—have this property, it is possible to postulate an interaction between a viral toxin and a chemical toxin. If other viruses, particularly those reputed to be associated with Reye's syndrome, also have such toxic properties this theory could have validity.

2) Simple virus infection allowing release of a stored chemical toxin. Virus infection could act as a releasing factor for stored chemical toxins. A similar phenomena is seen in children exposed to lead for a long period who develop acute encephalopathy after infectious contact (*17*).

3) Chemical enhancement of viral lethality by increasing replication and spread. Cell necrosis, however, has not been observed in the animal model and thus this hypothesis would have foundation only if the virus caused profound alterations in cell function without cell necrosis.

From 10 to 20 million gallons of petroleum oil by-products are used as pesticide dispersal agents and emulsifiers (*24*) each year in the United States alone. With this widespread use it is increasingly important to know the toxic nature of these chemicals, which are mainly ignored because they are considered safe on substantially insufficient grounds. The safety of these products is, of course, of increased importance in that such compounds are so widespread in our environment, being present in manufactured products other than insecticides. The effects in exposed humans may be cumulative, and the potential toxicity of these compounds could assume considerable significance both for inherent toxicity and enhancement of other agents of viral or toxic nature.

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Courtship Differences in Male Ring Doves: Avoidance of Cuckoldry?

Abstract. *Male ring doves exhibit less courtship and more aggressive behavior toward females that have recently associated with other males than to females that have been isolated. The difference in response may be related to the differing probability of cuckoldry.*

In many vertebrate species the female is most attractive to males at, or shortly before, ovulation. Moreover, at this time she is most likely to be receptive to their sexual advances. We have found, however, that male ring doves (*Streptopelia risoria*) court sexually unstimulated females more vigorously than they court females that are close to ovulation as a result of prior exposure to other males.

Trivers (*1*) suggests that, in those species in which the male contributes extensively to parental care, it is vital to the male that the eggs are fertilized by his own sperm. Otherwise, his large parental investment is wasted. If the male is attracted to and copulates with the female only at the time of ovulation, there is the possibility that she has been inseminated prior to his copulation. In polygynous and promiscuous species cuckoldry is of minor consequence since the male typically does not contribute to the reproductive effort beyond insemination, the cost of which, in energy and lost opportunities to mate with other females, is relatively small. However, when the male parental investment is large, mechanisms that ensure the genetic paternity of the investor increase in importance. Accord-

Table 1. Median performance levels and quartile deviations (Q) of males (N = 35) given 15 minutes with females that had been either exposed to other males (preexposed) or isolated for several weeks (unexposed).

Male behavior	Stimulus condition			
	Preexposed		Unexposed	
	Median	Q	Median	Q
Nest soliciting (duration in seconds)	90.0	112.8	185.0	182.6
Bowing and cooing (number of displays)	20.0	20.8	20.0	29.0
Chases (number of incidents)	18.0	25.5	10.0	7.7
Pecking (number of incidents)	12.0	13.3	3.0	3.4