Acute Toxicity Studies with Insect Attractants

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Acute Toxicity Studies with Insect Attractants. BEROZA, M., INSCOE, M. N., SCHWARTZ, P. H., JR., KEPLINGER, M. L. AND MASTRI, C. W. (1975). Toxicol. Appl. Pharmacol. 31, 421-429. Eight insect attractants and one inhibitor of a sex attractant were tested for acute oral and aerosol inhalation toxicity in rats, for acute dermal toxicity, eye irritation, and primary skin irritation on rabbits, and for toxicity to rainbow trout and bluegill sunfish. Except for phenethyl propionate + eugenol, 7:3 (acute dermal LD50, 1220 mg/kg), none of the test materials was classified as more than slightly toxic. (Z)-7-Dodecen-1-ol acted as a primary skin irritant, since it caused superficial chemical burns.

Insect attractants, both natural and synthetic, play a growing role in integrated insect pest control programs. For example, when insects of a target species are lured into a trap baited with an appropriate attractant, the catch indicates the presence and the whereabouts of the population, and this information can be used to restrict the application of control measures to those areas that require treatment and to times that will provide maximum benefit. Synthetic materials that were discovered empirically have been used in such monitoring and survey traps for some time, but the number of effective compounds is quite limited. Interest is currently focused on the increasing number of insect sex attractant pheromones that have been identified and made available synthetically during the past 8 years. These substances are produced by one sex to attract the opposite sex for mating; they are generally highly specific, and they are effective in survey traps at very low concentrations.

Some of the attractants are effective enough to show promise for the direct control of injurious insect species; they might be used for trapping, or the material could be released into the atmosphere to disorient mate-seeking efforts and thus prevent propagation (Beroza and Knipling, 1972). The amounts of material required in such applications are expected to be relatively minute. Effective traps may contain as little as 10 μg, and it is anticipated that applications in the range of 1–20 g/hectare (0.4–8 g/acre) would be used in the air-permeation method. The specificity of attractants (especially of the pheromones) should be of great value in such applications because only the target...
species would be affected. However we cannot proceed to large-scale field tests of attractants as means of control until we have enough information about their toxicity to assure us that they can be used safely. Accordingly, we summarize here toxicological data on nine chemicals (eight insect attractants and one compound that inhibits the action of a sex attractant) that show promise for use in insect control. Although attractants are generally not insecticidal, materials used for insect control are defined as pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended by the Federal Environmental Pesticide Control Act of 1972 (FEPCA), and toxicological data are needed for registration of compounds for which such use is contemplated.

The substances selected for study have been effective in laboratory and field tests against economically important insect pests (Table I). Three occur naturally as sex attractant pheromones: disparlure and looplure are emitted by the female gypsy moth and cabbage looper moth, respectively, to attract the male moths. Muscalure, produced by the female house fly, is attractive only to the male in laboratory tests but attracted both sexes in field tests (Carlson and Beroza, 1973). Hexalure, though not the natural pheromone of the pink bollworm, resembles a pheromone in its action. (Z)-7-Dodecen-1-ol inhibits the action of the cabbage looper pheromone (Tumlinson et al., 1972), and its dissemination may prevent the male from locating the female.

The other four test materials (cue-lure, methyl eugenol, PEP:eugenol (7:3), and trimedlure) do not appear to be related to any natural pheromones but have been found experimentally to be effective and specific attractants. The reported oral LD50 of methyl eugenol in rats is 1560 mg/kg (Jenner et al., 1964). Eugenol, a component of the Japanese beetle attractant, is the principal ingredient of oil of cloves; it has uses in perfumes and flavorings, and is used in dentistry as an antiseptic, an analgesic, and a component of dental cements. Its acute oral LD50 in rats was reported as 1930 mg/kg (Sober et al., 1950) and as 2680 mg/kg (Jenner et al., 1964; Hagan et al., 1965). Phenethyl propionate, the other component of the Japanese beetle attractant, is also used in perfumes and flavorings.

METHODS

The test materials are described in Table 1; they are colorless liquids that are soluble in the usual organic solvents. They were procured from commercial sources according to specifications set up by U.S. Department of Agriculture scientists; purity of each material was checked in the Organic Chemicals Synthesis Laboratory, AEQI, in Beltsville by gas chromatography. The limited amounts of material available for testing restricted the number of tests that could be made, particularly at higher dose levels.

Young albino rats of the Sprague-Dawley strain (150–240 g) were used in the tests of acute oral and inhalation toxicity. Albino rabbits of the New Zealand strain (1.9–3.1 kg) were used for the tests of acute dermal toxicity, eye irritation, and primary skin irritation. Healthy fingerlings (average length, 37–75 mm) of rainbow trout (Salmo gairdnerii) and bluegill sunfish (Lepomis macrochirus) were used in the tests of toxicity to fish (static fish toxicity studies). The rainbow trout were held at 13°C and the bluegills at 18°C.

3 Performed by Industrial Biotest Laboratories, Inc., under contract with USDA.
<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Purity (%)</th>
<th>Insect affected</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cue-lure</td>
<td>&gt;98</td>
<td>melon fly, Dacus cucurbitae (Coquillett)</td>
<td>Beroza et al., 1960</td>
</tr>
<tr>
<td>Disparlure</td>
<td>&gt;98</td>
<td>gypsy moth, Portheria dispar (L.)</td>
<td>Rierl et al., 1970</td>
</tr>
<tr>
<td>Hexalure</td>
<td>&gt;96</td>
<td>cabbage looper, Trichoplusia ni</td>
<td>Green et al., 1969</td>
</tr>
<tr>
<td>Looplure</td>
<td>&gt;96</td>
<td>cabbage looper</td>
<td>Berger, 1966</td>
</tr>
<tr>
<td>Looplure inhibitor</td>
<td>&gt;98</td>
<td>oriental fruit fly, Dacus dorsalis Hendel</td>
<td>Tuminson et al., 1972</td>
</tr>
<tr>
<td>Methyl eugenol</td>
<td>&gt;98</td>
<td>Japanese beetle, Popillia japonica</td>
<td>Steiner, 1952</td>
</tr>
<tr>
<td>Muscalure</td>
<td>&gt;96</td>
<td>Mediterranean fruit fly, Ceratitis capitata</td>
<td>Carlson et al., 1971</td>
</tr>
<tr>
<td>PEP:eugenol</td>
<td>&gt;98</td>
<td>Mediterranean fruit fly, Ceratitis capitata</td>
<td>McGovern et al., 1970</td>
</tr>
<tr>
<td>Trimedlure</td>
<td>&gt;98</td>
<td>Mediterranean fruit fly, Ceratitis capitata</td>
<td>Beroza et al., 1961</td>
</tr>
</tbody>
</table>
Acute oral toxicity. Albino rats. Food was withheld from the rats for 16 hr before dosing. Then, after an initial screening to determine the general range of toxicity, the undiluted liquid test material was administered at four, five, or six dose levels (to two male and two female rats at each level) directly into the stomachs with a hypodermic syringe that had a ball-tipped intubation needle. The rats were then placed individually in suspended wire-mesh cages and observed for 14 days. From the data the acute oral median lethal dose (LD50) of the test material was calculated (Weil, 1952; Thompson, 1947; Thompson and Weil, 1952). Necropsies were conducted on any animal that died during the study and on all animals that survived the 14-day observation period.

Acute dermal toxicity. Albino rabbits. The backs of the rabbits were shaved with electric clippers; the shaved area on each animal was about 30% of the total body surface. After a 24-hr waiting period to allow the stratum corneum to recover from any disturbance accompanying the close-cliping procedure and to permit healing of any microscopic abrasions, the undiluted liquid test material was applied, using two male and two female rabbits at each dose level. All materials were tested at 2025 mg/kg; PEP + eugenol and loopeplre inhibitor, which showed signs of greater toxicity, were tested at three and four additional dose levels, respectively. The site of application was covered by wrapping the trunk of the animal with plastic sheeting that was taped securely in place, and oral contact with the test material was prevented by fitting each animal with a light-weight flexible plastic collar that was worn throughout the observation period. When the test material had been in contact with the skin for 24 hr, the plastic sheeting was removed and all residues of test material were washed off, the test sites were examined for local skin reactions, and the animals were returned to their separate cages. Observations were continued for 14 days following the skin applications. Necropsies were conducted on all animals that died during the study and on all animals that survived the observation period.

Eye-irritation tests. Albino rabbits. The eye-irritating properties of each test material were determined by instilling a 0.1-ml dose of undiluted sample into the conjunctival sac of the right eye of each of six rabbits; the left eye of each animal served as the control. Grading for eye injury was made at 1, 24, 48 and 72 hr and at 7 days after treatment according to the grading and scoring system of Draize et al. (1944) in which a zero score indicates no irritation and the maximum score at any one scoring period is 110 (maximum irritation and damage to the cornea, iris, and conjunctiva).

Primary skin-irritation tests. Albino rabbits. The hair was clipped from the back and flanks of each of 6 rabbits, and two sites located lateral to the midline of the back and approximately 10 cm apart were selected for the test; one site was abraded by making four epidermal incisions, two perpendicular to the other two (Draize et al., 1944). A 0.5-ml dose of undiluted test material was applied to each site. The sites were immediately covered with 2-in.-square gauze patches that were secured with masking tape, and the trunk of each animal was wrapped with plastic sheeting to maintain the patches in position and retard evaporation of the test material. After 24 hr the plastic wrappings and gauze patches were removed, and the intact and abraded test sites were scored separately for erythema and edema on a graded scale of 0 to 4 (Draize et al., 1944); after 72 hr, the sites were scored again. The mean primary irritation score (maximum = 8) represents an average of the 24- and 72-hr ratings of the intact and abraded sites.
Inhalation toxicity tests. Albino rats. The tests were conducted in a 70-liter Plexiglass inhalation chamber designed to allow the introduction of the test animals into the test atmosphere after the maximum aerosol concentration was established. Ten rats, caged separately to minimize filtration of inspired air by animal fur, were exposed for one hour to the aerosol generated from the undiluted test material by a pneumatic nebulizer that produced 1- to 10-µm droplets. The average nominal concentrations of aerosol, the maximum attainable with the experimental equipment, were calculated by dividing the weight lost from the nebulizer by the total volume of air used and were as follows (in milligrams per liter air): cue-lure, 2.8; disparlure 5.0; hexalure, 3.8; looplure, 4.5; looplure inhibitor, 6.7; methyl eugenol, 4.8; muscalure, 26.6; PEP + eugenol (7:3), 5.0; and trimedlure, 2.9. Following exposure, the animals were observed for 14 days.

Static fish toxicity studies. For tests of toxicity to fish, bioassay vessels were lined with disposable polyethylene bags and filled with 12.5 liter reconstituted water (30 mg CaSO₄, 30 mg MgSO₄, 48 mg NaHCO₃, and 2 mg KCl added per liter of deionized water). Ten fish were placed in each vessel and allowed 24 hr to become acclimated. Calculated amounts of each test material, as 1% or 10% (w/v) solutions in acetone, were then added to the bioassay vessels. Each material was tested at five concentrations, selected on the basis of preliminary screening tests to determine the approximate ranges of toxicity. Control groups of fish in untreated water and in water to which acetone only was added were observed concurrently. The fish were observed for 96 hr and all deaths and/or untoward behavioral reactions were recorded. The concentration of dissolved oxygen was measured in all solutions in which deaths occurred to be sure the test water contained sufficient oxygen; dissolved oxygen concentrations above 4 mg/liter (4 ppm for the warm-water fish (bluegills) or above 5 mg/liter for cold-water fish (rainbow trout) were considered adequate. The median lethal concentrations (LC₅₀) of the seven test materials were calculated whenever the data permitted (Litchfield and Wilcoxon, 1949).

To check on suitability as test subjects, fish from each lot were challenged, under the same experimental conditions, with a reference pesticide (toxaphene, at 0.010, 0.018, and 0.056 ppm). The observed 96-hr LC₅₀ was usually around 0.02 ppm; with one lot of bluegills there were no deaths at 0.018 ppm and no survivors at 0.056 ppm.

RESULTS AND DISCUSSION

No deaths occurred and no adverse signs were noted in the inhalation toxicity tests; the calculated aerosol concentrations (the maximum concentrations which could be attained with the equipment used) ranged from 2.8 mg/liter air with cue-lure to 26.6 mg/liter air with muscalure.

Data from the other tests are summarized in Tables 2 and 3.

Acute oral toxicity, in the classification system used under the FIFRA, is categorized as follows: a material with an acute oral LD₅₀ of ≤50 mg/kg is highly toxic, one with an LD₅₀ of 50–500 mg/kg is toxic, one with an LD₅₀ of 500–5000 mg/kg is slightly toxic, and one with an LD₅₀ > 5000 mg/kg is nontoxic. Cue-lure, methyl eugenol, PEP: eugenol, and trimedlure are therefore classed as slightly toxic, while disparlure, hexalure, looplure, looplure inhibitor, and muscalure are nontoxic when administered orally. Disparlure, hexalure, and muscalure caused no deaths at the dose levels tested;
## TABLE 2

**Acute Toxicity of Eight Insect Attractants and an Inhibitor to Rat and Rabbit**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Acute oral LD50 (±SD) (mg/kg) (rats)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Acute dermal LD50 (±SD) (mg/kg) (rabbits)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Eye irritation score (rabbits)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Primary skin irritation score (rabbits)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/kg) (rats)</td>
<td>(mg/kg) (rabbits)</td>
<td>1 hr</td>
<td>24 hr</td>
</tr>
<tr>
<td>Cue-lure</td>
<td>3038 (+1266)</td>
<td>&gt;2025</td>
<td>12</td>
<td>1.3</td>
</tr>
<tr>
<td>Disparlure</td>
<td>&gt;34,600</td>
<td>&gt;2025</td>
<td>12</td>
<td>1.7</td>
</tr>
<tr>
<td>Hexalure</td>
<td>&gt;34,600</td>
<td>&gt;2025</td>
<td>8.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Looplure</td>
<td>&gt;13,430</td>
<td>&gt;2025</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Looplure inhibitor</td>
<td>&gt;11,730</td>
<td>&gt;2025</td>
<td>6.0</td>
<td>10.1</td>
</tr>
<tr>
<td>Methyl eugenol</td>
<td>1179 (+251.1)</td>
<td>&gt;2025</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Muscalure</td>
<td>&gt;23,070</td>
<td>&gt;2025</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>PEP: eugenol (7:3)</td>
<td>3980 (+ 957.8)</td>
<td>1220 (+189.6)</td>
<td>12</td>
<td>7.5</td>
</tr>
<tr>
<td>Trimedlure</td>
<td>4556 (+1136)</td>
<td>&gt;2025</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Dose or dose range (undiluted material) in brackets; four animals per dose. SD given (in parentheses) when it could be calculated.

<sup>b</sup> Draize scoring system; maximum score of 110 is the weighted summation of the grades for the three ocular tissues. Six rabbits per test; dose = 0.1 ml.

<sup>c</sup> Draize scoring system; maximum score is 8.0. Six rabbits per test, dose = 0.5 ml.

<sup>d</sup> No deaths at highest dose tested.

<sup>e</sup> Caused superficial chemical burns.

<sup>f</sup> One death at 2025 mg/kg.
**TABLE 3**

**TOXICITIES OF SIX INSECT ATTRACTANTS AND AN INHIBITOR TO FISH**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration range (ppm)</th>
<th>24 hr</th>
<th>48 hr</th>
<th>96 hr</th>
<th>Bluegill sunfish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hr</td>
<td>48 hr</td>
<td>96 hr</td>
<td>24 hr</td>
</tr>
<tr>
<td>Cue-lure</td>
<td>10-32</td>
<td>≥ 21</td>
<td>≥ 20</td>
<td>≥ 16</td>
<td></td>
</tr>
<tr>
<td>Disparlure</td>
<td>0.1-100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Looplure</td>
<td>0.1-100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Looplure inhibitor</td>
<td>1.8-4.4</td>
<td>3.7</td>
<td>3.7</td>
<td>3.7</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>(3.4-4.0)</td>
<td>(3.4-4.0)</td>
<td>(3.4-4.0)</td>
<td></td>
<td>(2.6-3.2)</td>
</tr>
<tr>
<td>Methyl eugenol</td>
<td>3.2-10.0</td>
<td>&gt;5.6, &lt;10.0</td>
<td>6.9</td>
<td>6.0</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>(5.5-8.6)</td>
<td>(4.9-7.2)</td>
<td></td>
<td></td>
<td>(7.4-9.0)</td>
</tr>
<tr>
<td>PEP:eugenol</td>
<td>10-32</td>
<td>≥ 20</td>
<td>≥ 14.5</td>
<td>&gt;10, &lt;13</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>(7:3)</td>
<td></td>
<td>(9.2-14.3)</td>
<td>(9.0-13.4)</td>
<td>(8.4-11.0)</td>
</tr>
<tr>
<td>Trimedlure</td>
<td>7.8-3.2</td>
<td>11.5</td>
<td>11.0</td>
<td>9.6</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td>(9.2-14.3)</td>
<td>(9.0-13.4)</td>
<td>(8.4-11.0)</td>
<td></td>
<td>(11.8-18.5)</td>
</tr>
</tbody>
</table>

* 95% confidence limits of LC50 (in parentheses) were determined when possible. Ten fish used at each concentration. Disparlure and looplure were tested at four concentrations, with no deaths occurring. Other compounds were tested at five concentrations.
they did evoke reactions such as hypoactivity, ruffed fur, diuresis, and slight muscular weakness, which lasted up to two days. Similar reactions were observed with low doses of the other materials, with additional indications of more severe generalized irritation and toxicity, such as labored breathing, hemorrhagic rhinitis and lacrimation, muscular weakness, and prostration, occurring with higher doses of those compounds. Necropsies of animals that died following administration of PEP: eugenol revealed pale spleens and gastroenteritis; no gross pathological alterations were noted in other animals.

For acute dermal toxicity the FIFRA categories are as follows: an LD50 \( \leq 200 \text{ mg/kg} \), highly toxic; an LD50 of 200–2000 mg/kg, toxic; an LD50 of 2000–20,000 mg/kg, slightly toxic. PEP: eugenol (dermal LD50 1220 mg/kg) is therefore classed as toxic and looplure inhibitor as slightly toxic (LD \( \approx 3700 \text{ mg/kg} \)). The other materials were not tested at concentrations higher than 2025 mg/kg, but since six of them caused no deaths and one (trimediure) caused one death at this dose level, they are all no more than slightly toxic. Ataxia, muscular weakness, and hypothermia were noted in the animals treated with looplure inhibitor. No untoward behavioral reactions were seen in any of the other animals. All the test chemicals caused local skin reactions characterized at the end of the 24-hr contact period by erythema and edema. With looplure inhibitor, focal second degree burns were noted at 24 hr. At the 7- and 14-day observations, no skin reactions were observed on animals treated with cue-lure; on the other animals, desquamation and pustulation were observed, with escharosis on the rabbits treated with disparlure and looplure inhibitor. Necropsies revealed no abnormal findings other than these dermal alterations.

For eye irritation, the FIFRA descriptive rating is assigned on the basis of the frequency, extent, and persistence of irritation or damage to the three ocular tissues. Thus, a test material is corrosive when the structure of the tissue at the site of contact is destroyed or changed irreversibly within 24 hr, is an irritant when four or more of six test animals show a positive reaction (Draize grade of 2 or more for any of the tissues) at any time between 24 and 72 hr, and is not an irritant if all average Draize grades are 1 or less. None of the test materials was an eye irritant.

For skin irritation, the categories are: a corrosive, if the intact skin is destroyed or changed irreversibly in 24 hr or less; an irritant, if the primary irritation score is \( \geq 5 \); not an irritant, if no irritation is observed or the primary irritation score is \( < 5 \). The only test material that was a skin irritant was the looplure inhibitor.

The most toxic substance in the static fish tests was the looplure inhibitor (LC50 for rainbow trout, 3.7 ppm; LC50 for bluegill sunfish, \( \sim 3 \text{ ppm} \)). No deaths and no adverse reactions were observed with disparlure and looplure. With the higher doses of cue-lure, PEP: eugenol, methyl eugenol, and looplure inhibitor, both species of fish became quiescent and flaccid, swimming or lying on their sides, with slow respiration. With trimediure, bluegills became flaccid, with shallow respiration, and lay on the bottom of the tank; with the trout, this compound evoked dark discoloration of the integument, rapid and shallow respiration, excessive swimming with gyration, and later lying on the bottom of the tank. Dark discoloration of the integument of trout was also observed with looplure inhibitor and with methyl eugenol.

In general, the nine materials tested had a low order of toxicity. In the small amounts that will normally be required in field work, the use of these chemicals should present no environmental problems from a toxicological standpoint.
REFERENCES


WEIL, C. S. (1952). Tables for convenient calculation of median-effective dose (LD50 or ED50) and instructions in their use. Biometrics 8, 249–263.