Mineral Oil and Aliphatic Alcohols: Toxicity and Analysis of Synergistic Effects on German Cockroaches (Dictyoptera: Blattellidae)

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ABSTRACT Two mineral oils and 12 linear primary alcohols were studied, alone and in combination, to determine their contact toxicity to adult German cockroaches, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). The more toxic oil, PD23 (LD_{50} = 1.45 mg per cockroach) was used for combination studies. Alcohols with carbon chain lengths of C3 and C8 through C12 were the most toxic, with LD_{50} values ranging from 0.3 to 0.6 mg. C1 (methanol) and C14 (1-tetradecanol) were least toxic, with LD_{50} values of 2.35 and 1.75 mg, respectively. Eight of the 12 combinations of a nonlethal dose of PD23 oil with an LD_{10} dose of alcohol produced significantly greater mortality than predicted under the assumption of additive effects. A sample of five synergistic oil + alcohol combinations, covering most of the alcohol carbon chain length range over which synergy occurred, was further studied by calculating LD_{50} values for three fixed mixture ratios (80:20, 50:50, and 20:80) of each combination. Results were analyzed using both graphical techniques (isobole analysis) and by nonlinear regression. At least one, but not necessarily all, of the three fixed ratio combinations of each oil + alcohol pairing indicated synergy. The conclusions drawn from the isobole and regression analyses were consistent.

KEY WORDS cockroach, oil, alcohol, toxicity, synergism

Insecticidal oils are commonly used for population suppression of soft-bodied plant pests such as aphids, mites, psyllids, scales, and whiteflies (Agnello 2002). Oils used alone can also control some relatively larger ornamental pest species (Baxendale and Johnson 1990). Maximum effectiveness of oil treatments requires direct contact with the insect or mite and sometimes repeated applications. Mineral oils, and other petroleum distillates, are often used as a component in aerosol formulations developed for the control of arthropod pests in urban environments. Mineral oils are permitted for use as "Inert Ingredients" in formulations of FIFRA 25(b) U.S. Environmental Protection Agency registration exempt pesticide products and the oils in these formulations can contribute to the effective control of pests while reducing the use of more toxic insecticidal ingredients. Alcohols are commonly used as solvents in insecticide formulations and as hard surface disinfectants, sanitizers, sterilants, virucides, fungicides, and mildewcides. Isopropanol (isopropyl alcohol) has had limited use as a topical treatment for controlling soft bodied insects such as mealybugs on ornamental plants, and it also has been used in combination with other pesticide active ingredients to kill fleas, ticks, and other household insects. Typically, aliphatic alcohols are not used as a stand-alone treatment for insect control but some studies and patents (Sinniah 1983, Cardin et al. 1994, Mougabure Cueto et al. 2002) indicate that alcohols can be effective against some medically important insects. Combinations of emulsifiers, adjuvants, and oils can result in enhanced insecticidal effects (Rae 2002), but there is little published information on combination effects of solvents, particularly alcohols, with oils.

In this report, we evaluated the contact toxicity of 12 aliphatic alcohols and two mineral oils, with different chemical and physical characteristics, to adult German cockroaches, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). We then studied interaction effects in the toxicity of oil + alcohol mixtures when the materials were simultaneously applied. Interaction effects were tested using two experimental procedures and analyzed using one graphic and two statistical methods.

Materials and Methods

Test Materials. Twelve primary aliphatic alcohols were evaluated. The names and chemical characteristics of these are shown in Table 1. All alcohols were obtained from Sigma-Aldrich (St. Louis, MO) and were of ≥98% purity. Two petroleum distillate mineral oils, PD-23 and PD-28, were obtained from Sonneborn LLC (Mahwah, NJ). The characteristics of these are shown in Table 2.

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Table 1. Chemical characteristics of linear aliphatic alcohols

Alcohol	Alkyl chain length	Molecular wt (g/mol)	Density (g/cm^3)	CAS no.	Water solubility at 25°C	Octanol/water partition coefficient (logP)
Methanol	C1	32.04	0.7918	67-56-1	Miscible	−0.56
Ethanol	C2	46.07	0.7890	64-17-5	Miscible	−0.05
1-Propanol	C3	60.10	0.8034	71-23-8	Miscible	0.42
1-Butanol	C4	74.12	0.8098	71-36-3	7.7 g/100 ml	0.95
1-Hexanol	C6	102.17	0.8136	111-27-3	0.59 g/100 ml	1.96
1-Heptanol	C7	116.2	0.8187	111-70-6	0.093 g/100 ml	2.46
1-Octanol	C8	130.23	0.8240	111-87-5	Insoluble	3.04
1-Nonanol	C9	144.26	0.8273	143-08-8	Insoluble	3.60
1-Decanol	C10	158.3	0.8297	112-30-1	Insoluble	4.05
1-Undecanol	C11	172.31	0.8298	112-42-5	Insoluble	4.48
1-Dodecanol	C12	186.34	0.8309	112-53-8	Insoluble	4.96
1-Tetradecanol	C14	214.39	0.8240	112-72-1	Insoluble	5.76

Test System and Effects of Individual Alcohols and Oils. Dose–response evaluations for each alcohol and oil were performed on 7–14-d-old adult male German cockroaches (SCJ strain, S.C. Johnson & Son, Racine, WI). Test arenas were 100- by 20-mm polystyrene petri dishes; the inside edge of each dish was lightly coated with mineral oil + petroleum jelly (1:3) to prevent cockroach escape. Cockroaches were anesthetized with a 15–25-s exposure to CO_2 and were then positioned with their ventral side up. Weight (milligram) amounts of individual or combinations of test materials were calculated and used for all tests. For alcohols (C12 and C14) that are solids at laboratory temperature (≈22°C), acetone was used as a solvent. A 1–3-μl drop of test material was applied to the area between the meso- and metathoracic legs by using a Rainin PR-2 or PR-10 pipette (Rainin Instrument, LLC, Oakland CA). Control cockroaches were treated with a 3 μl drop of 100% acetone (Sigma-Aldrich). Tests were evaluated after 24 h. Cockroaches were scored as either alive (dorsal side up, active movement when the abdomen was prodded with dissecting probe) or moribund (dorsal side up and no movement when abdomen prodded or ventral side up and insect unable to right itself)/dead. There were 12 cockroaches per replicate and at least three replicates (total $n \geq 36$) per treatment. Probit analysis (LeOra Software 2007) was used to calculate LD_{50} values and the associated 95% confidence intervals (CI).

Binary Mixture Effects: Primary Screen. An initial screen for studying activity of combinations of PD23 oil and alcohols was conducted by simultaneously applying a combination of a nonlethal dose of PD23 oil (0.40 mg) and an LD_{10} amount of alcohol. The LD_{10} alcohol dose was estimated from Probit analysis of the alcohol dose–response data. There were at least three replications (total $n \geq 36$) per treatment. The mortality in the 0.40 mg PD23 oil + LD_{10} alcohol combination treatments was compared with the mortality resulting from treatments with the individual components and the mortality of the untreated controls. Analysis of differences in the mortality data were performed using analysis of variance (ANOVA; SAS Institute 2009). Because one of the test compounds (oil) is inactive when used alone at a low dose, it becomes the potential synergist. Deviations in the percentage of mortality observed in the combinations of oil + alcohol compared with the alcohol alone are therefore attributed to the effects of the oil.

Table 2. Chemical and physical characteristics of two mineral oils tested on the German cockroach

	PD23 (CAS 8042-47-5)	PD28 (CAS 8042-47-5)
Specific gravity	0.800	0.823
Viscosity centistokes at 40°C	2.6	4.2
95% distillation range, °F	452–514	526–614
Molecular wt	220	245

Binary Mixture Effects: Isobolographic Analysis. A detailed analysis of interaction effects was made on a sample of five oil + alcohol combinations that produced significantly greater than expected mortality in the primary screen. The alcohols used were ethanol, 1-propanol, 1-butanol, 1-hexanol, and 1-octanol. Each combination of PD23 oil and alcohol was tested at three fixed ratios (weight:weight) representing relative proportions of the LD_{50} values: 20:80, 50:50, and 80:20. At least six concentrations of each fixed ratio blend were made and tested against adult male German cockroaches by using the procedures described above. LD_{50} values and 95% CIs for each blend ratio were calculated using Probit analysis. Isobolographic analysis, a graphic method often used in pharmacology to analyze mixture effects, was used to visually examine the LD_{50} values for the blends (Berenbaum 1989, Greco et al. 1995). This analytical approach has seen relatively little use in insect toxicology (Nelson and Kursar 1999). Combinations of A and B that produce the same effect (often the LC_{50} or LD_{50}) of A or B alone (the isoeffective dose combination) can be represented by a straight line on a two-dimensional isobolograph that connects, on the x and y axes respectively, the effective doses of A and B alone. This line has been termed the zero interaction isobole (Berenbaum 1989). If synergy is present, the amount needed by the combination to produce the same effect will be less than that for the individual components and the curve will seem to be "concave up" and bowing in. In con-

Table 3. Contact toxicity of mineral oils and primary linear alcohols to adult male German cockroaches

Test material	*n*	Slope (± SE)	χ^2	*P*	LD_{50} (95% CI)	LD_{10} (95% CI)
PD23-mineral oil	394	4.50 (0.36)	38.91 (30)	0.128	1.45 (1.30–1.63)	0.75 (0.63–0.86)
PD28-mineral oil	249	9.26 (1.20)	9.21 (10)	0.512	2.01 (1.92–2.11)	1.46 (1.29–1.59)
Methanol	151	n/a	n/a	n/a	>7.00	≈3.000
Ethanol	215	10.04 (1.29)	50.59 (15)	0.0001	2.36 (2.08–2.66)	1.76 (1.26–2.01)
1-Propanol	248	17.76 (2.03)	28.45 (13)	0.008	0.56 (0.53–0.58)	0.47 (0.41–0.50)
1-Butanol	143	19.81 (3.56)	7.51 (8)	0.483	1.02 (0.98–1.07)	0.88 (0.81–0.92)
1-Hexanol	157	6.39 (0.87)	4.03 (9)	0.909	0.72 (0.65–0.79)	0.45 (0.37–0.51)
1-Heptanol	154	5.50 (0.95)	11.73 (11)	0.380	0.79 (0.70–0.90)	0.46 (0.32–0.55)
1-Octanol	149	4.38 (0.72)	17.38 (10)	0.066	0.52 (0.39–0.64)	0.27 (0.12–0.40)
1-Nonanol	84	3.83 (0.76)	7.28 (5)	0.201	0.45 (0.30–0.63)	0.21 (0.05–0.31)
1-Decanol	272	6.93 (1.02)	37.15 (12)	0.0002	0.42 (0.37–0.48)	0.28 (0.15–0.33)
1-Undecanol	165	6.96 (1.01)	7.93 (11)	0.720	0.41 (0.37–0.44)	0.27 (0.22–0.30)
1-Dodecanol	135	7.81 (1.35)	14.99 (8)	0.059	0.36 (0.30–0.41)	0.25 (0.15–0.29)
1-Tetradecanol	131	7.76 (1.27)	8.98 (8)	0.344	1.75 (1.57–1.95)	1.19 (0.90–1.37)

LD_{50} and LD_{10} amounts are in milligrams. LD_{10} amounts of alcohols were tested in combination with 0.40-mg dose of PD23 mineral oil (see Table 4).

trast, antagonism will produce a "concave down" isobole which bows out.

Binary Mixture Effects: Fixed (Separate Ray [SR]) Design. The SR model was fit using the NLMIXED procedure in SAS (O'Brien 2004, Straetemans et al. 2005). This model uses the modified Gauss–Newton nonlinear statistical minimization algorithm to fit binary dose–response curves along each of the individual rays estimating individual LD_{50}s along each ray. It provides a rigorous statistical test for interaction (synergy or otherwise) along each of the rays by noting any departures from a line in the individual LD_{50}s. As with isobolograph analysis, this model allows for potential synergy along one ray (dose combination ratio) and antagonism or independent action along another ray. Along each ray, a combination index parameter (κ) is calculated which is the ratio of the actual estimated LD_{50} along the ray to that expected assuming independent action; $\kappa < 1$ indicates synergy, $\kappa \approx 1$ indicates independent action, and $\kappa > 1$ indicates antagonism.

Results

Activity of Individual Oils and Alcohols. German cockroach responses to individual oil and primary alcohols are shown in Table 3. Alcohols with carbon chain length of C3 and C8–C12 were the most toxic, with LD_{50} values ranging from 0.3 to 0.6 mg. The least toxic alcohol, methanol, had an estimated LD_{50} value in excess of 7 mg. Ethanol (LD_{50} = 2.36 mg) and 1-tetradecanol (LD_{50} = 1.75 mg) were also significantly less toxic than the other alcohols. PD23 mineral oil (LD_{50} = 1.45 mg) was significantly more toxic than PD28 mineral oil (LD_{50} = 2.01 mg). The relatively greater toxicity of PD23 was correlated to lower specific gravity, viscosity, distillation temperature, and molecular weight (Table 2).

Binary Mixture Effects: Primary Screen. Results of exposing cockroaches to a sublethal dose of oil combined with an LD_{10} dose of alcohol are shown in Table 4. Combinations of oil and C1 (methanol) through C9 (1-nonanol) alcohols produced significantly greater than expected mortality. The C3 through C7 alcohol series (1-propanol, 1-butanol, 1-hexanol, and 1-heptanol) produced highly significant departures from expected mortality.

Binary Mixture Effects: Isobolographic Analysis. Results of the isobole analysis are presented in Fig. 1. Each experiment included the parent oil and alcohol compounds alone as well as the combinations. The solid line drawn between the individual compounds is the zero interaction isobole. The dotted lines surrounding the zero interaction isobole represent the 95% CI. The three nonaxis points in each figure indicate the concentration of the combination, plus 95% CI, producing 50% mortality. The results demonstrate that the oil + alcohol interaction effects are influenced by both the chemical characteristics of the alcohol and the mixture ratio. For the oil + ethanol mixture (Fig. 1a), the 80:20 (oil + ethanol) blend LD_{50} overlaps the 95% CI of the parent compounds and is interpreted as an additive interaction. The 50:50 and 20:80 (oil + ethanol) blends lie outside of the 95% CI and below the zero interaction isobole, and these ratios seem to exhibit synergy. For 1-propanol (Fig. 1b), the 80:20 (oil + 1-propanol) and 50:50 blends support additive effects, whereas the 20:80 blend is synergistic. For 1-butanol (Fig. 1c) the 80:20 (oil + 1-butanol) blend is additive, whereas the 50:50 and 20:80 blends are synergistic. The oil + 1-hexanol combinations (Fig. 1d) were synergistic at all three ratios tested. In contrast, the oil + 1-octanol 80:20 combination showed evidence for weak antagonism while the 50:50 ratio was synergistic and the 20:80 blend was additive (Fig. 1e).

Binary Mixture Effects: Separate Ray Analysis. The results of this model fit to each of the combinations considered in the previous section produced almost identical results to those given above (with only minor exceptions). These exceptions are the following: in contrast with the above-mentioned isobologram analysis, the SR model indicates 1) weak antagonism for 80:20 oil + ethanol, 2) weak synergy for the 50:50 oil + propanol, 3) significant antagonism for the 80:20 oil + octanol, and 4) synergy for 20:80 oil + octanol.

Table 4. Contact toxicity of mineral oil (0.40 mg) combined with an LD_{10} dose of 12 primary linear alcohols to adult male German cockroaches

Treatment	*n*	No. dead at 24 h	Proportion dead at 24 h
PD23 - mineral oil (0.40 mg)	91	0	0
Methanol (3.0 mg)	39	1	0.026
Methanol (3.0 mg) + PD23 (0.40 mg)	38	13	0.342*
Ethanol (1.25 mg)	37	1	0.027
Ethanol (1.25 mg) + PD23 (0.40 mg)	37	11	0.297*
1-Propanol (0.45 mg)	36	1	0.028
1-Propanol (0.45 mg) + PD23 (0.40 mg)	37	35	0.946**
1-Butanol (0.8 mg)	35	1	0.029
1-Butanol (0.8 mg) + PD23 (0.40 mg)	39	28	0.718**
1-Hexanol (0.4 mg)	36	3	0.083
1-Hexanol (0.4 mg) + PD23 (0.40 mg)	37	37	1.000**
1-Heptanol (0.4 mg)	36	1	0.028
1-Heptanol (0.4 mg) + PD23 (0.40 mg)	36	35	0.972**
1-Octanol (0.2 mg)	37	2	0.054
1-Octanol (0.2 mg) + PD23 (0.40 mg)	36	9	0.250*
1-Nonanol (0.2 mg)	41	1	0.024
1-Nonanol (0.2 mg) + PD23 (0.40 mg)	36	8	0.222*
1-Decanol (0.2 mg)	37	1	0.027
1-Decanol (0.2 mg) + PD23 (0.40 mg)	36	1	0.028
1-Undecanol (0.25 mg)	38	4	0.105
1-Undecanol (0.25 mg) + PD23 (0.40 mg)	37	1	0.027
1-Dodecanol (0.2 mg)	39	3	0.077
1-Dodecanol (0.2 mg) + PD23 (0.40 mg)	37	10	0.270
1-Tetradecanol (1.0 mg)	37	4	0.108
1-Tetradecanol (1.0 mg) + PD23 (0.40 mg)	36	2	0.056

*, $P < 0.05$ significance and **, $P < 0.001$ significance of the specific alcohol tested alone compared with the specific oil + alcohol.

Discussion

Alcohols, such as isopropanol, are often used as "other" ingredients in insecticide formulations such as aerosols (Burke 1997) and probably contribute to the contact toxicity. They are seldom used alone as active ingredients despite documented toxic effects on insects such as mosquitoes (Sinniah 1983), head lice (Mougabure Cueto et al. 2002), and hematophagous Hemiptera (Mougabure Cueto et al. 2005). The mode of insecticidal action of primary alcohols is poorly known, but in microorganism cells, alcohols cause membrane damage, denaturation of proteins, metabolic disruption, and cell lysis (McDonnell and Russell 1999). Aliphatic alcohol effects on the insect nervous system are related to the carbon chain length and lipophilicity of the alcohol (Thomas 1976). Alcohol toxicity to head lice increased with an increase in the number of carbon atoms in the alcohol and the insecticidal activity may have been related to penetration/disruption of the cuticle (Mougabure Cueto et al. 2002). In the current study, the relation of alcohol toxicity to alkyl chain length was complex. Alcohols with the shortest carbon chains, methanol (C1) and ethanol (C2), and the longest carbon chain, tetradecanol (C14), were the least toxic while the intermediate carbon chain alcohols (C6–C10) were most toxic. Refined petroleum oils are well known for their effectiveness in the control of insect, mite, and other pests of agriculture (Agnello 2002) and it is not surprising that a highly refined petroleum (=mineral) oil such as PD23 with low viscosity has insecticidal activity. PD23 and similar oils are commonly used as solvents and show high affinity for heavy oils, grease, tar, and waxes. These oils readily penetrate through insect cuticle and cause mortality by disrupting the function of central nervous system cells (Taverner 2002, Najar-Rodriguez et al. 2008, Stadler and Buteler 2009).

Combinations of certain alcohols and petroleum-based oils can be more toxic than either of the individual components (Hurst 1940, Wigglesworth 1941). Oils also can enhance the efficacy of pesticides when applied in mixtures (Webb and Green 1945, Metcalf 1948). Oil + pesticide synergy effects are most often observed when the oil acts as the carrier or diluent in low-volume sprays (Rae 2002). One possible reason for this apparent synergy is the enhancement of cuticular penetration by the oil.

Analysis of synergy interactions between insecticidal chemicals typically involve two materials, one of which is nontoxic (or nontoxic at the dose tested in combination) combined with a toxic material (Eagleson 1940, Tozzi 1998). Most synergy studies compare the toxicity of an insecticidal active ingredient alone to the combination of the active plus a metabolic inhibitor such as piperonyl butoxide. Results are typically expressed as a synergist ratio that is the effective dose (such as LD_{50} or LC_{50}) of the toxic component alone divided by the effective dose of the binary combination (Chadwick 1961). Studies of synergy involving combinations of chemicals both of which are toxic represent a special experimental situation. No simple screening procedures exist for evaluating the combined effects of two chemicals, both of which have insecticidal activity. Likewise, there is no standard statistical procedure for analyzing results of these

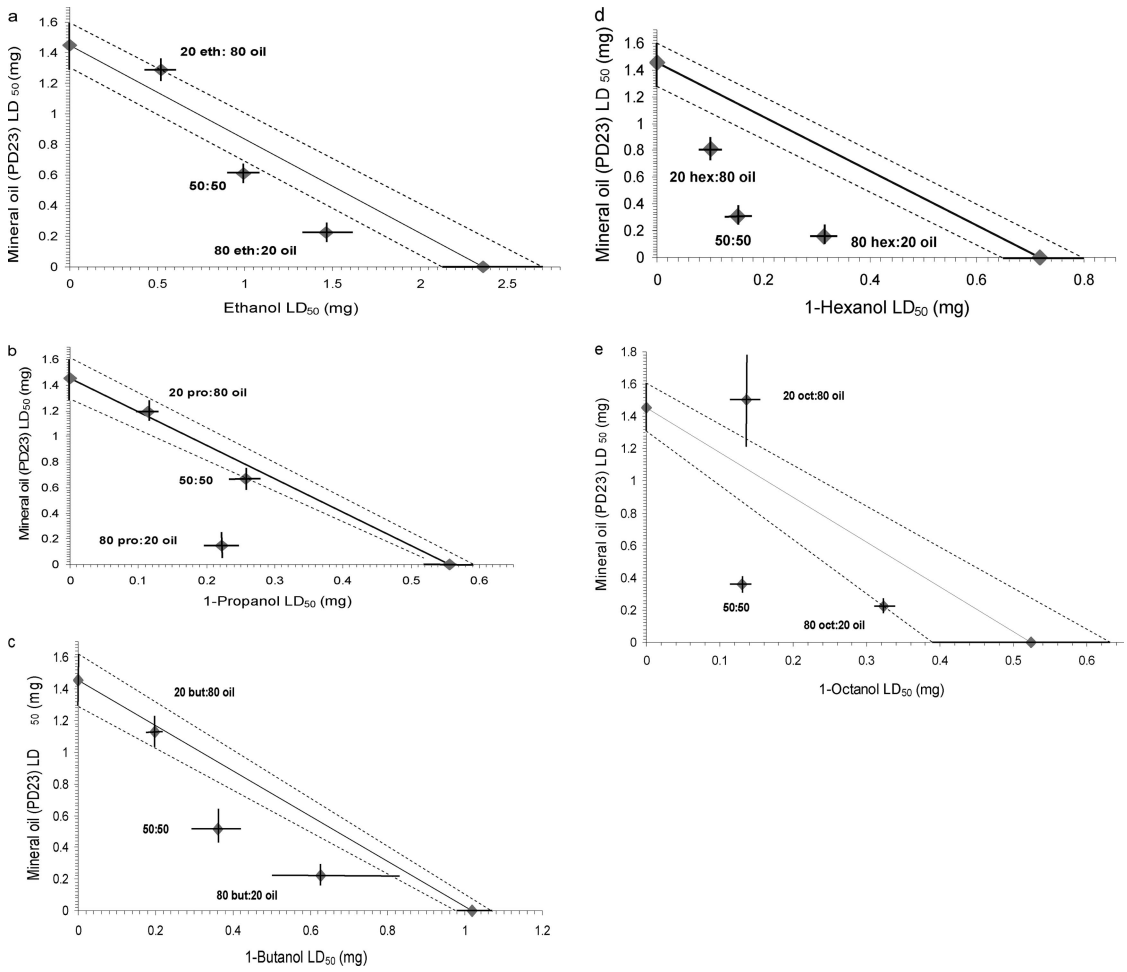

Fig. 1. Isobolographs showing mortality responses to combinations of alcohols and mineral oil. The additive isobole is the solid line connecting the zero interaction equieffective (LD_{50}) concentration of each alcohol and oil on the x and y axes. The 95% CI lines are drawn around the zero interaction isobole line. Each off-axis data point represents the LD_{50} (±95% CI) of an independent experiment using a fixed-ratio (20:80, 50:50, and 80:20) of the two ingredients.

combined effects (Nelson and Kursar 1999). In this study, we demonstrated that testing oil at a nontoxic dose combined with a slightly toxic dose (LD_{10}) of alcohol was a useful screen for identifying combinations with obvious synergistic effects. Results were initially analyzed using ANOVA. However, ANOVA may not be the optimal analytical method because it assumes that the data are linear, normally distributed, and with equal variances. Despite the possible shortcomings of ANOVA, it provided a quick way to screen pairs of active ingredients for interaction. Pairs showing greater than additive effects could then be validated by advanced testing. Subsequent determination of the LD_{50} values of three fixed ratios for each oil + alcohol combination provided data sufficient for graphical and advanced statistical analysis. Graphical isobologram analysis of drug interactions was introduced in the 19th century (Fraser 1870–1871, 1872). An isobologram is a Cartesian plot of pairs of doses that, in combination, yield a specified level of effect. Isobolograms are useful for graphically displaying results of drug combination and similar studies, because paired values of experimental points that fall below or above the line connecting the axial points (usually ED_{50} or LD_{50} values) denote supra- and subadditive combinations, respectively. However, because isobolograms do not fulfill the criteria for standard least squares regression analysis they are less useful for studying the entire range of combination ratios over which synergy may occur (Tallarida and Raffa 1995).

Statistical methods used to evaluate the combined action of two compounds are based on assumptions of their respective modes of action. Both the mineral oil and alcohols tested are functional solvents and may have a similar, but perhaps not identical, mode of action at target site(s). The Loewe additivity model (Loewe and Muischnek 1926) is based on the assumption that both compounds act, when combined, as if they were the same compound, i.e., they act in an additive manner. Similar mode of action supports the

use of the Loewe additivity model as a null hypothesis for evaluation of interaction between oils and alcohols. The Separate ray modeling methodology used here follows the Loewe approach (Straetemans et al. 2005). The model works best when the design is a so-called ray design—in this case, with three interior rays with slopes four (for the 80:20 mixture), one (for 50:50), and one-fourth (for 20:80). The SR model then simultaneously fits along each ray the binary logistic model:

$$\text{logit}(\pi) = \beta(z - \gamma) \qquad [1]$$

In this equation, logit is the usual logit formula, π corresponds to the probability of insect death, z is the effective dose of the two substances, and β and γ are the corresponding slope and LC_{50} parameters along the specified ray; maximum likelihood methods were used to estimate these parameters for the respective rays. In Fig. 1, the one-at-a-time LD_{50}s (the γ's in the equation correspond to the points on the horizontal and vertical axes, and the "independent-action" line corresponds to the down-sloping line connecting these two estimated points. If the estimated LD_{50} of an interior ray falls on or near this independent-action line, then this combination of the two substances behaves independently. If the LD_{50} falls closer to [further from] the origin, then synergy [antagonism] is indicated. For any two substances this model has the flexibility to detect different levels of synergy at different mixture ratios. Figure 1 illustrates this and shows that the synergy results are mixed for the 80:20 oil:alcohol mixtures, but the 50:50 and the 20:80 mixtures show significant synergy for each of the alcohols.

In summary, we found that a screening approach using a nontoxic dose of oil combined with a low level toxic dose (LD_{10}) of alcohol was effective for initial identification of synergistic interactions. More advanced graphical and statistical analysis of the interaction effects were required to demonstrate that synergy does not necessarily occur at every fixed ratio. In all of the fixed ratio tests, the strongest synergistic effects were typically produced by the 20:80 oil + alcohol combination.

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