Nordgren et al. (1981) estimated that dichlorvos was eliminated much faster than trichlorfon ($t_{1/2}$ trichlorfon = 690 min; $t_{1/2}$ dichlorvos = 0.65 min). Brandal (1979) found residues of trichlorfon in muscular tissue of Atlantic salmon up to 12 days after treatment with 300 ppm for 60 min. A concentration of approximately 0.7 μg/g was detected 1 day after treatment. In the present study, the maximum dichlorvos concentration found in muscular tissue directly after treatment with 2 ppm for 60 min was 0.13 μg/g. The decline in dichlorvos concentrations in muscular tissue to nondetectable levels within 1–3 days after treatment was as expected, assuming that in salmon, as in man, the elimination of dichlorvos is faster than the elimination of trichlorfon.

In conclusion, dichlorvos is rapidly excreted in Atlantic salmon. The withdrawal time of 14 days set by the Norwegian Medicines Control Authority thus has a built-in safety margin which is sufficient to ensure that no residues are present in muscular or liver tissues in fish at slaughter.

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Insecticidal and Antifeedant Bioactivities of Neem Oils and Their Relationship to Azadirachtin Content†

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Neem seed oil, a possible precursor to new botanical insecticides, varies widely with respect to concentrations of the limonoid azadirachtin, the putative active ingredient. Among 12 samples of neem oil analyzed by liquid chromatography, azadirachtin concentrations ranged from <50 (limit of detection) to over 4000 ppm. Oils were bioassayed for larval growth inhibition and antifeedant activity against the variegated cutworm (*Peridroma saucia*) and for molt disrupting activity against the milkweed bug (*Oncopeltus fasciatus*). For each of the three bioassays, bioactivity of the oils, as measured by EC_{50} values, is highly correlated with azadirachtin content of the oils. From 72% to 90% of the variation in bioactivity of the oils can be accounted for by variation in azadirachtin content. As azadirachtin content of neem oil varies widely and is highly correlated to bioactivity against these bioassay species, azadirachtin content may be a useful quality-control criterion for neem oil as a precursor for insecticide production.

INTRODUCTION

Many current neurotoxic insecticides are damaging to the environment and/or pose a threat to public health via food residues, groundwater contamination, or accidental exposure. Although the risks associated with the proper use of these pest control materials should be minimal, the exaggerated perception of these risks among the public is increasing demand for alternative or "organic" produce, which necessitates development of new, environmentally sound pest control materials. A potential source of new pesticidal materials are higher plants; the search for new insecticides or prototypes among plant natural products has heightened in the past decade (Arnason et al., 1989; Balandrin et al., 1985).

One such source of natural insecticides is the neem tree (*Azadirachta indica* A. Juss.; Meliaceae). Native to the Indian subcontinent, this fast-growing shade tree has been widely cultivated in Africa, Australia, the Carribean, and Central and South America. Although the seeds and leaves of this tree have been traditionally used for centuries to control pests (Koul et al., 1990), recent interest in neem

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as a crop protectant dates to the work of Pradhan et al. (1962), who reported that dilute seed extracts completely prevented feeding of the desert locust, *Schistocerca gregaria*. The major active principle, azadirachtin (AZA), a ring C-seco tetranortriterpenoid, is the most potent natural insect antifeedant discovered to date. More recently, AZA has been demonstrated to strongly interfere with molting and reproduction in several species of insects (Koul et al., 1987; Koul, 1984; Sieber and Rembold, 1983; Pener et al., 1988), which points to the neuroendocrine system as a target site. Thus, AZA is dually advantageous as a natural insect control agent because it possesses both protectant (antifeedant) and toxic (insect growth regulatory) properties against insects. Over 200 species of pest insects in seven orders are known to be susceptible to the bioactivity of azadirachtin (Saxena, 1989), representing over 90% of the species so far tested.

The lack of acute toxicity in lab animals (oral LD_{50} in rats $\gg$5000 mg/kg) and lack of evidence for chronic effects in animals, combined with a long historical use of neem preparations in traditional medicine in India (Koul et al., 1990), should make a neem-based insecticide a highly acceptable alternative to synthetic neurotoxin-type insecticides.

The complex chemical nature of AZA and the diverse structural requirements for insect bioactivity (Yamasaki and Klocke, 1987; Rembold, 1989) are barriers to the synthesis of this molecule or bioactive analogues, although such efforts are currently underway (Ley et al., 1987). However, because relatively crude extracts or derivatives of neem seeds or leaves have been demonstrated to be effective in field trials (Schmutterer and Ascher, 1984, 1987), development of a commercial insecticide based on an AZA-rich neem preparation appears feasible (Larson, 1989). Advantages of neem preparations over pure AZA include the presence of other potentially active constituents (Kraus et al., 1987; Balandrin et al., 1988) and the possibility that a botanical preparation may enhance the stability of AZA and other actives. At present, development of insecticides based on AZA-fortified neem extracts or derivatives is underway on at least three continents.

One derivative of neem which may serve as a starting point for the development of an insecticide is the oil obtained from crushing of the dried seed. Neem seed, which contains approximately 40% oil, has the highest concentrations of AZA in the plant. India currently produces over 80 000 metric tons of neem oil annually, which is used primarily in the manufacture of soap. Crude aqueous emulsions of neem oil are efficacious against insect pests in field trials (Olaifa and Adenuga, 1988; Chiu, 1989), which suggests that the crude oil may be a useful starting material for the development of a neem-based insecticide.

Neem, like all other botanical preparations, will vary considerably with respect to concentrations of its active principles depending on the genome of the tree(s) from which seed is collected, the geographic area of origin, and yearly variations in environmental conditions. The objective of the present study was to assess the degree to which AZA content varies between commercially provided samples of neem oil from different sources in India and to determine the relationships between AZA content of oils and biological activity, in terms of both antifeedant (behavioral) efficacy and growth regulatory (physiological) efficacy, by using separate bioassay organisms.

MATERIALS AND METHODS

Insects. Variegated cutworm larvae (*Peridroma saucia* Hubner) (Lepidoptera: Noctuidae) were obtained from a laboratory colony reared on artifical diet (BioServ Inc., Frenchtown, NJ, No. 9795) supplemented with finely ground alfalfa meal to improve its acceptability to neonate larvae. Nymphs of the large milkweed bug (*Oncopeltus fasciatus* Dallas) (Hemiptera: Lygaeidae) were obtained from a laboratory colony reared on dried seeds of *Asclepias speciosa* Torrey, collected annually at Kelowna, BC. Both colonies were maintained in an insectary at 24 °C and 16:8 LD.

Chemicals. Azadirachtin (>95% purity based on FAB–mass spectroscopy) was isolated from Indian neem seeds (*A. indica* A. Juss.) by the modified Nakanishi method (Schroeder and Nakanishi, 1987). Neem oils (i.e., produced by crushing dried seeds under pressure; also referred to as "expeller" oil) were provided by Safer Ltd. (Victoria, BC), who in turn obtained them from various commercial sources in different locations in India in 1987 and 1988. The actual sources of the oil samples remain proprietary information.

Analysis of Azadirachtin in Oils. Azadirachtin (AZA) content of neem oils was determined by reverse-phase HPLC following a simple cleanup procedure. One gram of crude oil was added to 10 mL of 50% aqueous MeOH and 10 mL of diethyl ether. The phases were mixed by vigorous shaking and then allowed to separate overnight. The aqueous phase was removed for analysis (first wash) and the ether phase reextracted with 10 mL of 50% aqueous MeOH (second wash). The first extraction normally contained 75–85% of the total AZA recovered from an oil sample; a third extraction of the organic phase failed to yield measurable amounts of AZA in several trials with the most AZA-rich oils.

The HPLC system consisted of a Waters Model 640 chromatograph with a Model 490 multiwavelength UV detector. Samples were resolved on a 3.9 mm × 15 cm Novapak C_{18} column 4-μm particles) using a linear gradient of 30–48% aqueous acetonitrile in 6 min at a flow rate of 1.0 mL/min. Absorbance was measured at 210 nm. Using this system, azadirachtin has a retention time of 4.5 min, with a limit of detection of approximately 50 ppm in oil (0.05 μg/g). For quantitation of AZA in neem oils, three injections were averaged for each wash and values for the two washes added to give the final values reported in Table II.

To determine the accuracy of analysis, 1.0-g samples of three oils were fortified with either 0.5 or 1.0 mg of pure AZA and then subjected to cleanup and HPLC analysis. Overall recovery of AZA from the spiked samples averaged 86%.

Chronic Larval Growth Bioassay. Test diets (20 g) containing neem oils or pure azadirachtin were prepared as follows. The test substance was added to 5 mL of water containing 0.1% Triton X-100 as an emulsifier, and the resulting emulsion was added to the agar solution (gelling agent) and dry diet ingredients after heating. After cooling, the diet was cut into 20 pieces that were placed individually into 30-mL plastic cups to which were added two neonate cutworm larvae (within approximately 12 h of hatching). The cups were placed in plastic boxes lined with water-soaked paper towels to maintain high humidity, and the boxes were held in an environmental chamber at 27 °C and 16:8 LD. After 9 days, all larvae were weighed, and the mean weights for each treatment group were expressed as a percentage of controls. For each oil, six dietary concentrations were tested along with a control.

Antifeedant Choice Bioassay. Antifeedant activity of neem oils was assayed by using a leaf-disk choice test (Koul and Isman, 1990). Disks (3.5 cm^2, average fwt = 117 mg) were punched out from cabbage leaves (*Brassica oleracea* var. Stonehead) and treated on each side with 15 μL of aqueous neem oil solutions emulsified with 0.1% Triton X-100. Zero concentration disks were treated with 15 μL of the carrier alone. After the solutions had dried, two fifth instar larvae were introduced into each arena (a 10-cm diameter plastic Petri dish) containing one treated and one control disk. The Petri dishes were held at ambient (21 °C) temperature in complete darkness for 5 h. The consumption of leaf material was recorded by using a digitizing leaf area meter (LiCor Model LI-3000). The index of feeding deterrency for each treatment was calculated as $(C - T)/(C + T) \times 100$, where C is the consumption of control disks and T the consumption of treated disks. For each oil, four concentra-

Table I. Azadirachtin Content and Bioactivity of Neem Oils from Various Sources in Behavioral and Physiological Insect Bioassays

sample code	azadirachtin content, ppm	*Peridroma* chronic growth EC_{50} (95% CI), ppm in diet	*Peridroma* choice test EC_{50} (95% CI), $\mu g/cm^2$	*Oncopeltus* molting inhibition ED_{50} (95% CI), μg/nymph
SCR/52/18	ND[a]	$\gg$600[b]	$\gg$30.0[b]	$\gg$50.0[b]
SCR/53/23	ND	$\gg$600[b]	28.0[c]	50.0[c]
SCR/50/11	188	610 (500–740)	17.7 (15.5–20.2)	41.0 (31.3–53.8)
RSB/1/166/3	214	410 (270–640)	25.1 (20.4–30.9)	40.1 (31.9–50.5)
SCR/49/6	491	180 (120–260)	4.9 (3.5–6.9)	11.5 (8.6–15.4)
SCR/50/1	629	430 (270–680)	5.7 (4.0–8.1)	30.0 (24.4–36.9)
SCR/50/9	712	210 (160–280)	7.4 (5.8–9.5)	29.0 (24.7–34.1)
RSB/1/166/1	853	190 (150–250)	6.0 (4.3–8.4)	20.0 (17.9–22.4)
SCR/50/8	1084	240 (190–310)	6.7 (5.4–8.3)	10.0 (7.3–13.6)
RSB/1/166/2	2884	60 (40–80)	2.1 (0.6–2.6)	1.5 (1.2–1.8)
RSB/1/166/4	2990	70 (50–90)	2.5 (0.5–3.2)	1.5 (1.3–1.8)
SCR/50/3	4026	50 (30–70)	2.0 (1.5–2.6)	1.1 (1.0–1.2)

[a] ND = none detected; detection limit approximately 50 ppm in oil. [b] Maximum concentration or dose tested; no activity observed at this dose. [c] 50% effect observed at this concentration or dose, but higher doses not tested.

tions and a control were tested with five replicates per treatment.

Molting Inhibition Bioassay. Molting inhibition bioactivity of neem oils was assessed following topical application to newly molted (within 24 h) fifth instar milkweed bug nymphs. Although not economically important, this species is frequently used in investigations of putative insect growth regulators, and its sensitivity to AZA has previously been reported (Dorn et al., 1986; Champagne et al., 1989). Test solutions were applied to the abdominal dorsum in 1 μL of acetone, while controls were treated with the carrier alone. Bugs were kept in 10-cm diameter Petri dishes, provided with milkweed seed (*A. speciosa*) and a cotton dental roll soaked with water. The dishes were placed in an environmental chamber at 27 °C and 16:8 LD and monitored daily after 6 days for molting to the adult stage. Controls normally molted 7 days after treatment, whereas molting in treated bugs, if it occured at all, was usually delayed by 3–4 days. For our study, molt inhibition was defined as any deviation from molting to a morphologically normal adult, i.e., from deformity of the wings and/or legs to death during molting. For each oil, 4–5 doses and a control were tested, with 15 nymphs per treatment. For each of the bioassays described, concentration ranges of the respective oils were assigned on the basis of a preliminary series of bioassays in which all of the oils were compared at a single concentration.

Data Analysis. For each oil, means for each concentration, expressed as percent of larval growth relative to controls, percent feeding deterrence, or percent successfully molted, were subjected to probit analysis (Finney, 1971). For each bioassay, EC_{50} values for each oil, determined by probit analysis (dependent variables) and log transformed to assure random distribution of residuals, were correlated to AZA concentration in the oils as determined quantitatively by HPLC.

AZA-Spiked Oil Bioassays. A further set of bioassays was conducted to compare bioactivity of AZA to bioactivity of a zero-AZA oil (SCR/52/18) spiked with pure AZA. For each of the three bioassays, four or five concentrations of pure AZA or the spiked oil were tested. In the case of the chronic growth bioassay, AZA was added to the oil at a fixed concentration (2000 ppm), and increasing aliquots of the oil were added to the artificial diet. To test the hypothesis that AZA is the only active component of neem oil, these treatments (pure AZA, AZA-spiked oil) were compared to three high-AZA oils (RSB/1/166/2, RSB/1/166/4, and SCR/50/3) added to diets in proportional amounts such that each treatment was bioassayed at concentrations of 0.1–0.5 ppm AZA equivalents. For the choice test and molting inhibition bioassays, the amount of oil was held constant (28.6 $\mu g/cm^2$ and 50 μg/nymph, respectively) with increasing amounts of AZA added. These quantities of oil were representative of the highest concentration or dose tested that had no biological effect in the initial set of bioassays. All dependent variables were log transformed, and linear regression was used to define dose–response relationships. An F-test was used to test equality of the two regression lines (intercepts) for each bioassay, and a t-test was used to compare regression slopes of AZA with regression slopes for the AZA-spiked oil (Neter et al., 1985).

RESULTS

Quantitative analysis of neem oils by HPLC indicated that AZA content varied widely between samples (Table I). Two of the 12 oils did not contain detectable levels of AZA (detection limit = 50 ppm), while the remaining oils contained from 188 to 4026 ppm. It is noteworthy that two of the three high-AZA oils were from the same supplier in India, but based on oils produced in consecutive years (RSB/1/166/2 and SCR/50/3). However, two samples provided from a different supplier produced in consecutive years differed markedly in AZA content (RSB/1/166/4 and SCR/50/9).

Each of our bioassays shows a clear trend in which bioactivity of neem oils is related to AZA content. Of the two oils lacking detectable AZA, one was inactive even at the highest concentration or dose tested, and the other had minimal activity at the highest concentration tested (Table I). Among oils with detectable amounts of AZA, the two with the lowest contents were consistently the least biologically active, whereas the three oils containing 3000–4000 ppm were consistently the most active in all three bioassays.

Overall, the molting inhibition bioassay with *Oncopeltus* nymphs has the highest correlation with AZA content of neem oils ($r = -0.95$, $p < 0.0001$), although the two bioassays with *Peridroma* larvae, the chronic growth bioassay and the antifeedant bioassay (choice test), are both highly correlated with AZA content ($r = -0.92$, $p < 0.0001$ and $r = -0.85$, $p < 0.001$, respectively) (Figure 1).

Comparing bioactivity of pure AZA to a zero oil spiked with AZA in the *Peridroma* chronic growth bioassay suggests that bioactivity of AZA is enhanced in the presence of the oil (Figure 2). Linear regression analysis of the log-transformed data indicates that the intercepts differ significantly ($p < 0.05$), whereas the slopes do not, suggestive of an additive effect of the oil. Intercepts for each of the neem oil dose–response curves differ significantly from that of pure AZA, and each oil produces a slope which differs significantly from that of pure AZA (Figure 2). The steeper slopes of the dose–responses for the high-AZA oils, compared to those of the AZA-spiked zero oil or pure AZA, suggest the presence of other constituents in those oils which synergize or activate AZA.

For the other two bioassays (choice test and molting inhibition) the oil does not appear to have any influence on the bioactivity of AZA (Figures 3 and 4). Neither the regression slopes nor intercepts differ significantly ($p = 0.05$) between regression lines for pure AZA and AZA-spiked oil in either bioassay.

EC_{50} values for pure AZA in each of the three bioassays are presented in Table II.

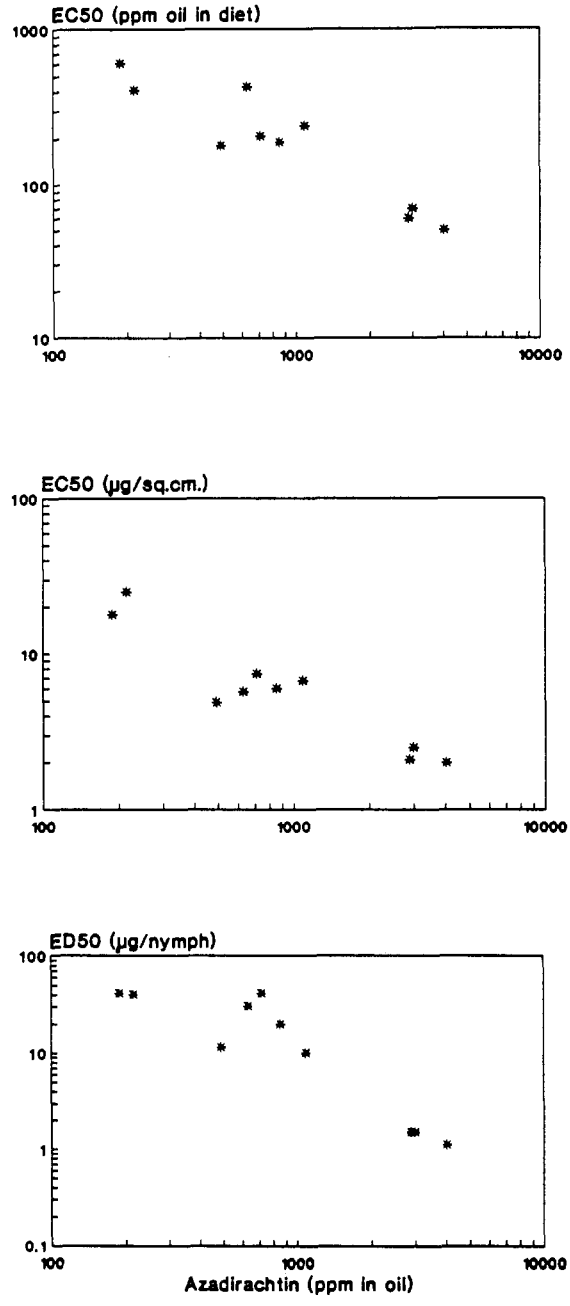

Figure 1. Scattergrams of bioactivities of neem oils (EC_{50} or ED_{50}) versus azadirachtin content of oils. (Top) *Peridroma* chronic growth bioassay; (middle) *Peridroma* leaf-disk choice test; (bottom) *Oncopeltus* molt inhibition bioassay.

DISCUSSION

AZA is well-known for its insect antifeedant and growth regulating actions [reviewed in Saxena (1989)]. Neem oil, in which AZA is the proposed principal active ingredient, has demonstrated efficacy as a crop protectant against several insect pests (Schmutterer and Ascher, 1984, 1987, and references cited therein). In the present study, we have confirmed that AZA is largely responsible for both antifeedant (behavioral) and growth-regulating (physiological) activities of neem oils, utilizing bioassays specific to each action. In the case of the chronic growth bioassay, AZA may be exerting an overall effect through both behavioral and physiological means. The salient point is that the strong correlations between bioactivities of the oils and their AZA contents suggest that variation in AZA can account for most (72–90%) of the variation in activities ascribed to the oils, at least with respect to the bioassay species we employed.

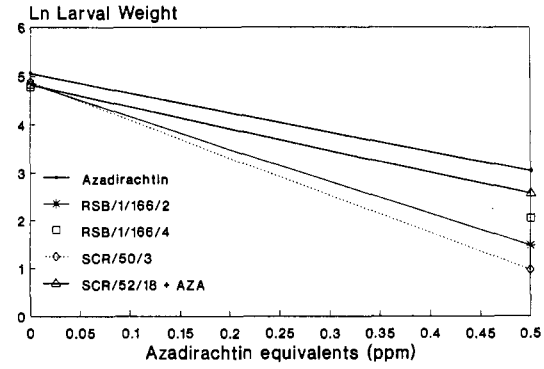

Figure 2. Regression lines for log larval weight of neonate *P. saucia* reared on artificial diets containing pure AZA, a zero-AZA neem oil to which AZA was added, or three different high-AZA neem oils versus dietary azadirachtin equivalents. SCR/52/18 alone had no appreciable bioactivity at the highest concentration tested.

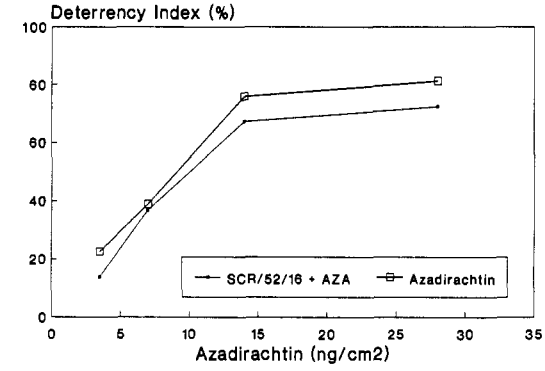

Figure 3. Deterrency of pure AZA and a zero-AZA neem oil spiked with AZA in the leaf-disk choice test using fifth instar *P. saucia* larvae. SCR/52/18 alone had no appreciable bioactivity at the highest concentration tested.

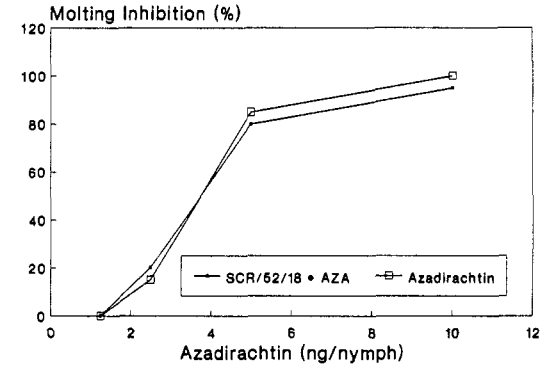

Figure 4. Inhibition of molting in *O. fasciatus* fifth instar nymphs treated topically with pure AZA or a zero-AZA neem oil spiked with AZA. SCR/52/18 alone had no appreciable bioactivity at the highest dose tested.

Table II. Efficacy of Pure Azadirachtin in Three Insect Bioassays

bioassay	EC_{50} (95% CI)
neonate *Peridroma* chronic growth	0.26 (0.18–0.38) ppm
fifth instar *Peridroma* feeding choice test	8.0 (4.4–14.4) ng/cm^2
fifth instar *Oncopeltus* molting inhibition	3.5 (2.9–4.1) ng/nymph[a]

[a] Average weight of nymphs at time of treatment = 25 mg fwt.

Other potential actives in the oil include numerous limonoids, both structurally similar (Rembold, 1989b) and dissimilar (Aranson et al., 1987) to AZA, and disulfides (Balandrin et al., 1988). This last group of compounds

are volatile and contribute to the strong, garlic-like odor of neem oil and have demonstrated growth inhibitory action against larvae of *Heliothis* species when added to artificial media (Balandrin et al., 1988). However, the major volatile constituent of crushed neem seeds, di-*n*-propyl disulfide, has dietary EC_{50}s of 850–940 ppm against *Heliothis*, compared to the EC_{50} for AZA of 0.07 ppm in this species (Yamasaki and Klocke, 1987).

Rembold and co-workers (Rembold, 1989a) have isolated seven AZA isomers from neem seeds and evaluated their molt-disrupting activities against the Mexican bean beetle *Epilachna varivestis*. Their AZA A (equivalent to the pure AZA used in our study) constitutes almost 83% of the total AZAs isolated from neem seeds, and their AZA B, which is slightly more active, constitutes a further 16% of the total. Therefore, the contribution of the five remaining AZA analogues (less than 1% of the total), and perhaps even that of AZA B, to the total bioactivity of neem oil is probably insignificant relative to that of AZA A. Similarly, even though other limonoids from neem and related Meliaceae have demonstrated bioactivity against insects [e.g., Champagne et al. (1989)], none of these are within 2 orders of magnitude as active as AZA, and thus their contribution to bioactivity of neem oils may be largely discounted.

One explanation for the apparent enhancement of AZA activity in the presence of neem oil in the artificial media (chronic growth) bioassay could be that the oil somehow facilitates bioavailability of the AZA molecule to receptors in the chemosensillae of *P. saucia* larvae, relative to the bioavailability of pure AZA in the aqueous medium of the normal artificial diet.

A similar analysis of the relationship between AZA content of neem and bioactivity has been reported by Ermel et al. (1987) on the basis of the efficacy of enriched neem seed extracts using the *Epilachna* molt inhibition bioassay. In their study, pure AZA had an EC_{50} of 0.33 ppm, while the average estimated amount of AZA at the EC_{50} for five extracts was 0.328 ppm. EC_{50}s of the extracts were highly correlated to log AZA concentrations ($r = -0.95$), but it should be pointed out that AZA concentrations in their concentrated extracts ranged from 1.4% to 20.9%, whereas the AZA concentrations in the neem oils tested in the present study ranged from 0.2% to 0.4%.

The correlation coefficients for bioactivity versus AZA content obtained from our bioassays suggest that from 72% to 90% of the variation in bioactivity of neem oils can be accounted for by AZA conent. Ermel et al. (1987) concluded from their study that AZA is the major active principle in neem seeds, and on the basis of our results, we concur with this conclusion.

Neem seeds from different geographic regions are known to vary considerably with respect to AZA content (Ermel et al., 1987) and bioactivity (Singh, 1987). AZA content of neem oils is highly variable depending on the source of the seeds from which it is prepared, and AZA content is highly correlated with both behavior-disrupting and insect growth-regulating activities of oils, on the basis of our bioassay species. However, there are undoubtedly other insect species for which this correlation is not significant, for example, the strawberry aphid, *Chaetosiphon fragaefolii* Cockerell (D. T. Lowery and M. B. Isman, unpublished data). Nonetheless, we suggest that AZA content should constitute a useful quality-control criterion for the acceptability of neem oil as a precursor for a formulated botanical insecticide. The most practical approach in future should be to attempt to correlate AZA content of neem oils with biological activity by using species against which the resulting commercial products are intended or registered. Results of our bioassays further suggest that neem oils containing AZA at levels of 2000 ppm or greater should have sufficient bioactivity for their utilization in the preparation of neem-based insecticides.

ABBREVIATIONS USED

AZA, azadirachtin; EC, effective concentration; FAB, fast atom bombardment; HPLC, high-performance liquid chromatography.

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In Vitro Studies of the Metabolism of Atrazine, Simazine, and Terbutryn in Several Vertebrate Species

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The metabolism of three triazine herbicides (atrazine, simazine, and terbutryn) was studied in rat (Sprague-Dawley and Fischer strains), mouse, goat, sheep, pig, rabbit, and chicken by using in vitro hepatic 10000*g* supernatant or microsomal systems. Principal phase I metabolites were 4- or 6-monodealkylated-*s*-triazines; several observations, including studies with purified enzymes, demonstrated that phase I reactions were cytochrome P-450 mediated. There were species-related variations in rates of metabolism and in ratios of primary metabolites, although no strain- or sex-related differences were noted. Phase II products were glutathione conjugates of the parent compound and of the two monodealkylated products. Experiments with Fenton's reagent, which generates hydroxyl radicals, gave dealkylated 2-chloro-*s*-triazine, supporting the possible role of active oxygen radicals in the cytochrome P-450 mediated reactions.

Atrazine, simazine, and terbutryn are chemically similar, symmetrical or *s*-triazine herbicides. The dominant phase I metabolic reaction (reaction adding a reactive polar group) for triazine herbicides in animals is N-dealkylation (Bohme and Bar, 1967; Crayford and Hutson, 1972; Dauterman and Muecke, 1974; Khan et al., 1979; Erickson et al., 1979; Bradway and Moseman, 1982), and the primary phase II reactions (biological conjugations yielding products that are more water soluble than the unconjugated material) are conjugations with glutathione and with glucuronides (Guddewar and Dauterman, 1979; Dauterman and Muecke, 1974; Crayford and Hutson, 1972; Larsen and Bakke, 1978). 2-(Methylthio)-*s*-triazines, such as terbutryn, have also been shown to form the 2-hydroxyl and the 4-(aminobutyl) alcohol or 4-(aminobutyl) carboxylic acid (Larsen and Bakke, 1978; and Larsen et al., 1978). Because of their persistence in the environment (Parmeggiani, 1983) and their wide-

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