Biological activity and acetylcholinesterase inhibition by nonfumigant nematicides and their degradation products on *Meloidogyne incognita* (1)

Dieter NORDMEYER* and Don W. DICKSON

Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611-0611, USA.

SUMMARY

The biological activity of ethoprop, oxamyl, fenamiphos, fenamiphos sulfoxide, fenamiphos sulfone, aldicarb, aldicarb sulfoxide, aldicarb sulfone, carbofuran, 3-hydroxy-carbofuran and 3-keto-carbofuran were determined for *Meloidogyne incognita* by calculating the EC_{50} (nematicide concentration that reduced root galling by 50 %) for galling on tomato plants. The EC_{50} values for each compound were 0.7, 0.3, 0.2, 0.2, 0.3, 0.5, 0.5, 0.9, 2.3, 8 and 5 µg/ml, respectively, whereas their I_{50} values (nematicide concentration that inhibited acetylcholinesterase activity by 50 %) were 875, 0.17, 555, 58.2, 22.5, 3.1, 0.14, 5.38, 0.02, 0.37 and 0.59 (1×10^{-6} M), respectively. Comparisons of the EC_{50} values with their respective I_{50} values showed little correlation between *in vivo* and *in vitro* activity of the compounds. Different rates of degradation of the parent compound and the nature and nematicidal activity of their metabolites might be responsible for this discrepancy.

RÉSUMÉ

Activité biologique contre Meloidogyne incognita *et inhibition de l'acétylcholinesterase par des nématicides non volatils et par leurs produits de dégradation*

L'activité biologique des composés ethoprop, oxamyl, fenamiphos, fenamiphos sulphoxyde, fenamiphos sulphone, aldicarbe, aldicarbe sulphoxyde, aldicarbe sulphone, carbofuran, 3-hydroxy-carbofuran et 3-keto-carbofuran contre *Meloidogyne incognita* a été mesurée en déterminant les concentrations réduisant de 50 % 1) la formation de galles sur les racines de tomate (EC_{50}) et 2) l'activité de l'acétylcholinesterase (I_{50}). Les valeurs respectives obtenues sont, pour EC_{50} : 0,7; 0,3; 0,2; 0,2; 0,3; 0,5; 0,5; 0,9; 2,3; 8 et 5 µg/ml et pour I_{50} : 875; 0,17; 555; 58,2; 22,5; 3,1; 0,14; 5,38; 0,02; 0,37 et 0,59 (1×10^{-6} M). La comparaison des valeurs EC_{50} et I_{50} montre que la corrélation entre les activités *in vivo* et *in vitro* d'un même composé est faible. Cela peut être dû à un taux de dégradation du composé de base différent *in vivo* et *in vitro*, au type des métabolites produits, et à leur activité.

There is general agreement that the toxic action of organophosphate and carbamate pesticides upon nematodes, insects and vertebrates is caused by their ability to inhibit acetylcholinesterase (AChE) in various parts of the nervous system, and thereby, disrupt nervous transmission at that location (Corbett, Wright & Baillie, 1984). Acetylcholine is an impulse transmitting substance that bridges the gap of cholinergic synapses. The enzyme AChE is responsible for the destruction of the transmitter. When AChE is inhibited, acetylcholine accumulates at the postsynaptic membrane which leads to continuous stimulation and to paralysis of the organism.

The degree of AChE inhibition and biological activity of these compounds do not always correlate, however, as was shown for the nematode *Aphelenchus avenae*, insects and other organisms (Metcalf, 1971; Stenersen, 1979*a*, 1979*b*; Pree, Towshend & Archibald, 1987). Our objective was to determine whether the inhibition of root galling by nonfumigant nematicides and their degradation products was related to their ability to inhibit the AChE of *Meloidogyne incognita* (Kofoid & White) Chitwood.

Materials and methods

The nonvolatile nematicides and their degradation products used in our experiments are listed in Table 1. The activity of these chemicals in suppressing *M. incog-*

(1) Florida Agricultural Experiment Station Journal Series No. 9522.

* *Current address : Ciba-Geigy, Ltd., Basel, Switzerland.*

Table 1

Effect of various nonfumigant nematicides and their degradation products in reducing galling of tomato roots caused by *Meloidogyne incognita* and the inhibition of the nematode's acetylcholinesterase (AChE).

Chemical	EC_{50} µg/ml[a]	I_{50} AChE (1×10^{-6} M)[b]
Fenamiphos	0.2	555
— sulfoxide	0.2	58.2
— sulfone	0.3	22.5
Aldicarb	0.5	3.1
— sulfoxide	0.5	0.14
— sulfone	0.9	5.38
Oxamyl	0.3	0.17
Ethoprop	0.7	875
Carbofuran	2.3	0.02
3-hydroxy-	8.0	0.37
3-keto-	5.0	0.59

(a) Nematicide concentration that reduced root galling by 50 %.
(b) Nematicide concentration that inhibited 50 % of the nematode AChE.

nita infection was expressed as EC_{50} for root galling (nematicide concentration that reduced root galling by 50 %). This parameter was determined by using the glass vial method described by Bunt (1975). Two hundred freshly hatched second-stage juveniles of *M. incognita* were exposed to different concentrations of each nematicide or their degradation products (4, 1, 0.25, and 0.063 µg/ml) in sandfilled glass vials. The nematicides used were technical formulations. Stock solutions (1 000 µg/ml) of each chemical was prepared in acetone and stored at 5 °C no longer than 14 days. A tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) seedling was then transplanted into the vial and placed in a growth chamber at 25 °C for 10 days. After this period galls that had developed were counted and the EC_{50} for each treatment was calculated.

In order to relate the biological activity of the nematicides used with their ability to inhibit AChE, 250 000 second-stage juveniles of *M. incognita* were homogenized in ice-cold sodium phosphate buffer (0.1 M, pH 7.5) in a glass tissue grinder with glass beads (0.1 mm diameter). Samples were left in the buffer for 24 h, at 5 °C to improve solubilization of membrane bound AChE (Johnson & Russell, 1983). Then the samples were centrifuged at 80 000 *g* in a Beckman Airfuge for 20 min at 5 °C. The clear supernatant was used for AChE determination.

Acetylcholinesterase assays were routinely carried out at room temperature (25 ± 2 °C) by a slight modification of the single-vial radiometric method of Johnson and Russel (1975). Reaction volumes of 100 µl in a 1-dram scintillation vial contained 70 µl HEPES buffer (25 mM, pH 7.0), 10 µl clear supernatant from the nematode homogenate (enzyme), 10 µl inhibitor (nematicide), and 10 µl [3_H] acetylcholine (2×10^{-5} M). Assays were initiated by enzyme addition and stopped after 10 min at room temperature by the addition of 100 µl of a " stopping solution " containing 1 M monochloroacetate buffer (pH 3.0) and 2.0 M NaCl. This was followed immediately by addition of 4.0 ml of scintillation mixture (0.5 % PPO, 0.03 % POPOP in toluene — 10 % vol/vol 1-butanol), capping and shaking of the vials and counting in a liquid scintillation counter. Duplicates of each sample were assayed. Each set of assays included several blanks and complete conversions (with electric eel AChE) as controls. All counts were corrected for background and quenching. The I_{50} (nematicide concentration that inhibited 50 % of the nematode AChE) was determined by assaying different concentrations of each nematicide or their degradation products. The degree of inhibition was determined in percent and the I_{50} values was calculated for each treatment.

Results

There was little difference in the biological activity of fenamiphos, aldicarb, oxamyl, ethoprop, and their degradation products in reducing galling of tomato roots, however, carbofuran and its two major metabolites were clearly less effective (Table 1). When comparing the EC_{50} values for galling of the individual chemicals with their respective I_{50} values for AChE inhibition, no relationship between the two parameters could be shown. The organophosphates fenamiphos and ethoprop inhibited the nematode AChE the least. In contrast, carbofuran and its metabolites showed a much greater suppression of AChE activity (Table 1). Carbofuran was about 30 000 and 40 000 times more inhibitory than fenamiphos or ethoprop, respectively. Both organophosphates required considerably higher concentrations for inhibiting nematode AChE than did the three carbamates.

The sulfoxidated metabolites of fenamiphos and aldicarb were 10 to 22 times more inhibitory than their parent compounds. The sulfone was more inhibitory than the sulfoxide metabolite of fenamiphos to the nematode AChE; whereas, for aldicarb the sulfone metabolite was less inhibitory than the parent compound or the sulfoxide metabolite (Table 1). This *in vitro* activity correlates with the slight decrease in the biological activity of aldicarb sulfone compared to its parent compound. A similar relationship was observed for the carbofuran metabolites. Their higher I_{50} values were accompanied by decreased biological activity.

Discussion

There was no relationship between the *in vivo* and *in vitro* activity comparing different nematicides; however, comparisons between parent compound and their metabolites show somewhat better relationships for these two parameters. These data suggests that the nematicidal efficacy of fenamiphos and aldicarb in the field may be due to sulfoxidation of the parent compound. This hypothesis is supported by the fact that both parent compounds have a rather short half-life in water and in soil (Waggoner & Khasawinah, 1974; Smelt *et al.*, 1978) and are also probably metabolized rapidly within the nematode itself. Also, results from Cuany *et al.* (1981) support this hypothesis for aldicarb.

Oxamyl was very inhibitory to the nematode AChE, however, the half-life of oxamyl is reported to be rather short in plants and soil and the rapid rate of its metabolism always leads towards ineffectiveness of the compound (Harvey & Han, 1978; Harvey, Han & Reiser, 1978). This would indicate a decrease in nematicidal activity corresponding to the rapid degradation of the parent compound.

The discrepancy of the substantial *in vivo* activity of ethoprop and its low ability to inhibit nematode AChE is not readily explained. It is possible that ethoprop is metabolized by nematodes into a biologically more active compound. In insects, ethoprop is metabolized into a more potent metabolite (Wing, Glikman & Casida, 1984). Since no ethoprop metabolites were available to us the possibility of increased biological activity of the compound via breakdown of the metabolites could not be explored. The low *in vivo* activity of carbofuran stands in contrast to its high affinity for the nematodes AChE. Recent results have shown that in nematodes carbofuran is rapidly converted to watersoluble metabolites (> 90 %) other than 3-hydroxy-carbofuran or 3-keto-carbofuran (Nordmeyer *et al.*, 1989). Although carbofuran is an extremely potent inhibitor of nematode AChE, its effectiveness as a nematicide is greatly reduced by detoxification.

Our finding that fenamiphos is a poor AChE inhibitor, whereas, aldicarb, oxamyl, and carbofuran are much more potent is supported by data from Pree, Townshend and Archibald (1987) on AChE from *Aphelenchus avenae*. Difference in efficacy of nonfumigant nematicides observed in the greenhouse or in the field cannot be fully explained solely on their inhibitory effects on AChE. In addition, recent studies with *Caenorhabditis elegans* demonstrate that AChE is distributed in nonnervous tissue indicating functions other than interruption of acetylcholine signal transmission (Johnson *et al.*, 1988). Other factors such as physicochemical parameters like lipophilicity, water solubility, adsorptivity, volatility, and stability of nematicides and their degradation products will also have an impact on their efficacy influencing their mobility in soil and plant.

ACHNOWLEDGEMENTS

We thank DuPont, FMC, Mobay, Mobil, and Union Carbide for providing technical formulations of nematicides and metabolites and partial support of this investigation.

REFERENCES

BUNT, J. A. (1975). Effect and mode of action of some systemic nematicides. *Meded. Landbouwhogeschool Wageningen*, 75 : 1-128.

CORBETT, J. R., WRIGHT, K., & BAILLIE, A. C. (1984). *The biochemical mode of action of pesticides. Second Edition.* London, Academic Press, 382 p.

CUANY, A., BERNIER, F., BRIDE, J. M., WALLACH, J., & BRIDE, J. B. (1981). Caractérisation des ChE chez deux nématodes : Action inhibitrice de l'aldicarbe et de ses dérivés d'oxydation. *Trav. Soc. Pharmacie Montpellier*, 41 : 147-151.

HARVEY, J. & HAN, J. C. Y. (1978). Decomposition of oxamyl in soil and water. *J. Agric. Food Chem.*, 26 : 536-541.

HARVEY, J., HAN, J. C. Y., & REISER, R. W. (1978). Metabolism of oxamyl in plants. *J. Agric. Food Chem.*, 25 : 529-536.

JOHNSON, C. D., RAND, J. B., HERMANN, R. K., STERN, B. D., & RUSSELL, R. L. (1988). The acetylcholinesterase genes of *C. elegans* : identification of a third gene (*ace*-3) and mosaic mapping of a synthetic lethal phenotype. *Neuron*, 1 : 165-173.

JOHNSON, C. D. & RUSSELL, R. L. (1975). A rapid, simple radiometric assay for cholinesterase, suitable for multiple determinations. *Ann. Biochem.*, 64 : 229-238.

JOHNSON, C. D. & RUSSELL, R. L. (1983). Multiple molecular forms of acetylcholinesterase in the nematode *Caenorhabditis elegans*. *J. Neurochem.*, 41 : 30-46.

METCALF, R. L. (1971). Structure-activity relationships for insecticidal carbamates. *Bull. World Health Org.*, 44 : 43-77.

NORDMEYER, D., DICKSON, D. W., OU, L. T., & CROMROY, H. L. (1989). Uptake, accumulation, and metabolism of carbofuran and fenamiphos by the phytoparasitic nematodes *Meloidogyne javanica* and *M. incognita*. *Pestic. Biochem. & Physiol.*, 34 : 179-184.

PREE, D. J., TOWNSHEND, J. L., & ARCHIBALD, D. E. (1987). Sensivity of acetylcholinesterases from *Aphelenchus avenae* to organophosphorous and carbamate pesticides. *J. Nematol.*, 19 : 188-193.

SMELT, H. J., LEISTRA, M., HOUX, N. W. H., & DECKER, A. (1978). Conversion rates of aldicarb and its oxidation products in soils. III. Aldicarb. *Pestic. Sci.*, 9 : 293-300.

STENERSEN, J. (1979*a*). Action of pesticides on earthworms. I. The toxicity of cholinesterase-inhibiting insecticides to earthworms as evaluated by laboratory tests. *Pestic. Sci.*, 10 : 66-74.

STENERSEN, J. (1979*b*). Action of pesticides on earthworms. III. Inhibition and reactivation of cholinestereases in *Eisenia foetida* (Savigny) after treatment with cholinesterase-inhibiting insecticides. *Pestic. Sci.*, 10 : 113-122.

WAGGONER, T. B. & KHASAWINAH, A. M. (1974). New aspects of organophosphorous pesticides. VII. Metabolism, biochemical, and biological aspects of nemacur and related phosphoroamidate compounds. *Residue Reviews*, 53 : 79-97.

WING, K. D., GLICKMAN, A. H., & CASIDA, J. E. (1984). Phosphorothiolate pesticides and related compounds : Oxidative bioactivation and aging of the inhibited acetylcholinesterase. *Pestic. Biochem. & Physiol.*, 21 : 22-30.

Accepté pour publication le 20 septembre 1989.