

SOT CONFERENCE AT NASHVILLE, TENNESSEE¹

Derek Gammon of the California Department of Pesticide Regulation reports on this year's conference of the Society of Toxicology held from 17–21 March 2002

Neuroreceptors as the basis for insecticide resistance and selective toxicity

Resistance of insects to several insecticides was described in this symposium, organized by J. Casida (U.C. Berkeley) and T. Narahashi (Northwestern Univ., Chicago).

K. Dong (MSU) reviewed mutations in Na channel genes in the German cockroach giving rise to populations with a range of pyrethroid susceptibilities. The molecular biology of resistance to several classes of insecticide was described by R. French-Constant (Univ. Bath, UK). Resistance to cyclodienes, such as dieldrin, due to an altered GABA receptor gene, has been found to extend cross-resistance to fipronil. A single gene mutation resulting in Ala→Ser has been identified in multiple species of cyclodiene-resistant insects, all over the world. More practical considerations concerning resistance prediction were also discussed. Although not included in the symposium, it is worth reporting that the prize for the best publication in *Toxicology & Applied Pharmacology* during 2001 was awarded to John Casida, G. S. Ratra and S. G. Kamita. Their paper addressed the issue of differences between receptor subunit binding of a GABA_A receptor ionophore antagonist (³H]-EBOB) to recombinant human GABA_A receptor subunits. The GABA_A receptor is the target site for several important classes of insecticide, including the cyclodienes, lindane, endosulfan and fipronil. In the symposium, M. Tomizawa and Casida described the receptor binding characteristics of imidacloprid to various nicotinic ACh receptors. Such nitromethylene analogs of nicotine are considerably more toxic to insects than to mammals, the opposite being the case for nicotine itself. It appears that the analogs that are selective against insects are unprotonated whereas those that are toxic to mammals are ionized at blood pH. It was proposed that the former compounds, possessing a nitro or nitrile group, interacted with a cationic site in the nACh-R whereas in the latter case, the iminium cation interacts with an anionic site in the nACh-R. Electrophysiological studies of imidacloprid action on voltage-clamped cockroach neurones revealed some interesting features (Salgado, Aventis, Frankfurt). Imidacloprid has previously been found to be an agonist at the insect nACh-R, but V. Salgado found both agonist and antagonist effects. Iontophoresis of ACh resulted in two distinct (inward) currents, a large early one that desensitized rapidly and a smaller, later one that did not desensitize. Imidacloprid

blocked the former (IC₅₀ 2.4 nM) and activated the latter (EC₅₀ 700 nM). Both currents were blocked by α-Bungarotoxin but with different IC₅₀ values. It was concluded that there were two types of nACh-R in the cockroach, thus adding an element of uncertainty to some previous interpretations of receptor binding data at the insect nACh-R.

Safety assessment of foods derived from biotech food crops

In a workshop organized by J. Borzellaca (Medical School of VA) and G. Burdock (Consultant), Burdock himself pointed out that within 50 years, there will be *ca.* 3-fold more food needed on earth, from essentially the same land area. There was thus a need to increase productivity without jeopardizing the safety of food. There is a desire in many influential circles for the adoption of the "Precautionary Principle," whereby any modified food should be considered dangerous until it had been thoroughly tested. Burdock and other speakers felt that, with the tremendous amount that had been learned about GM food toxicity over the past few years, that "Substantial Equivalence" could be claimed for all GM foodstuffs tested to date. This is also the position of the SOT, according to a draft position paper on this subject.

A. Brynjolfsson (Wayland, MA) described the history of efforts to convince the public and regulators that irradiated food was safe. Irradiation has been used to sterilize food for improved storage characteristics for over 50 years. In 1948, the US Army began studies to establish the safety of such foods. However, it was not until the 1980s or 1990s that irradiated foods became generally recognized as wholesome. Even now, some sectors of society believe, incorrectly, that there is a connection between irradiated food and nuclear contamination. Various features of GM food should help to avoid some of the problems associated with irradiated food acceptability. For example, DNA is not inherently toxic or allergenic and indeed, is classified in the US as GRAS (generally recognized as safe). According to I. Munro (CANTOX, Canada) the average person consumes several hundred mg of DNA per day, without reported toxicity. Furthermore, it is broken down rapidly by DNAases and there is negligible incorporation of DNA in the diet into human cells. It was also pointed out that food itself is not inherently "safe" and so the best standard to set for GM food is that there is "a reasonable certainty of no harm." Many of the safety tests conducted on GM food involve comparing the amino acid sequence to known allergens.

¹ The views expressed in this report represent those of the author and not necessarily those of Cal/EPA.

Endocrine effects

Triazine herbicide toxicity in mammals

M.E. Anderson's group (CO State Univ.) assessed the ability of atrazine to inhibit the binding of estradiol (E_2) to receptors in the hypothalamus in the SD rat. In earlier experiments, atrazine has been found to suppress the LH surge in female SD rats. In the undosed rat, LH is released by the pituitary following the stimulation of E_2 *alpha* receptors in the hypothalamus, an example of positive feedback. In the experiments described, atrazine inhibited the binding of E_2 to these receptors *in vitro*, dose-dependently, with an IC_{50} of $\sim 0.3 \mu M$, without requiring prior incubation. However, binding inhibition could not be shown *in vivo* after dosing rats at levels that resulted in suppression of the LH surge. This could have been due to the inherent difficulties of conducting *in vivo* hormone receptor-binding assays using inhibitors that are not irreversible. Thus, the inhibition of E_2 binding to the *alpha* receptor in the hypothalamus by triazine herbicides remains a plausible explanation of the suppression of the LH surge, which is considered to be the basis for the increased incidence of mammary tumors in the female SD rat after chronic triazine administration. Another plausible explanation for some of the "endocrine" effects of atrazine is the induction of aromatase (CYP19), an enzyme that converts androgens to estrogens. The group of J. T. Sanderson (Utrecht, NL) continued their research in this area, showing that whereas triazine herbicides are potent inducers (EC_{50} 2-5 μM), imidazole fungicides, such as imazalil, are potent inhibitors (IC_{50} 0.1 μM) of aromatase in H295R cells (from a human adrenocortical carcinoma cell line). Imazalil appeared to act through a mixed competitive/non-competitive mechanism. Triazole fungicides, such as propiconazole, were also inhibitors (IC_{50} 5 μM) of aromatase activity, acting competitively. The mechanism of induction appeared to involve an increased level of cyclic AMP, possibly through an inhibition of phosphodiesterase activity. The aromatase enzyme is becoming increasingly important because it is a key target site for the treatment of E_2 -sensitive mammary tumors in humans. This enzyme is also critical for successful pregnancy maintenance.

S. D. Turner *et al.* (BD Biosciences, Woburn, MA) measured the potency of inhibitors using a recombinant CYP19 assay and found the results to be comparable to a human placental microsome CYP19 assay. Certainazole fungicides, that may be taken by pregnant women, were potent inhibitors of aromatase.

Methoxychlor endocrine effects

E. Muroso and R. Derk (CDC, Morgantown, WV) showed that both methoxychlor and its desmethylated, insecticidal metabolite inhibited the biosynthesis of testosterone in rat Leydig cells. The inhibition, occurring at 1 – 1000 nM, did not appear to be due to an interaction at an estrogen or androgen receptor. Instead, it seems that the P_{450} enzymes responsible for cholesterol side-chain cleavage are inhibited.

Cumulative exposure to pyrethroids

L. Sheets (Bayer, KS) reported on the possibility that pyrethroids may undergo a "cumulative" risk analysis by USEPA under FQPA. This refers to the possibility of exposure to multiple chemicals with a common mechanism of toxicity. It has recently been applied to limited sets of organophosphate insecticides, each acting through the inhibition of AChE. However, for the pyrethroids, it is generally accepted that there are two types of action, the Type I Syndrome (also known as the "T" syndrome because it is associated with tremors in dosed rodents) and the Type II Syndrome (also known as the "CS" syndrome, since it includes choreoathetosis and salivation in rodents).

Furthermore, several nerve channel target sites have been identified in insects, crustacea and mammals. Possible sites of action have been considered by an industry-wide task force, with the addition of two academics, D. Soderland (Cornell Univ., NY) and J. Clark (Univ. MA, Amherst). These include three voltage-sensitive ion channels in nerve membrane, for Na, Ca and Cl ions, plus the peripheral type benzodiazepine receptor (for Ro5-4864). It was argued by the task force that the multiplicity of possible target sites (pleiotropism) made it impossible to develop a meaningful "cumulative risk" model to address pyrethroid toxicology. The relative importance of the four sites (above) was being addressed largely through the model of potency of pyrethroids against various ion channels inserted into *Xenopus* oocytes, under voltage clamp.

However, it is not unreasonable to expect that particular pyrethroid target sites may be responsible for specific clinical signs in experimental animals. Moreover, these target sites may be in different conformational states in isolation compared with their natural state, perhaps making the use of ion channels in oocyte membranes an overly simplistic representation of pyrethroid effects *in vivo*. It therefore seems that there could be much work to do to address one of the most difficult questions in toxicology: how do effects recorded *in vitro* manifest themselves *in vivo*?

IN THE NEXT ISSUE

It is hoped that the next issue of *Pesticide Outlook* will include articles on such diverse topics as:

- Sulfonylureas
- Insect antifeedants
- Residues in food
- Organic farming
- Slugs