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Mammalian toxicity and safety evaluation *

by

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Toxicological studies on the insecticides submitted to WHO are intended to supplement and not to replace work that is the responsibility of the manufacturer.

At the earliest stage, it is desirable to try to discover whether the new insecticide will be safe to apply as a conventional indoor residual spray in malaria control (FRANCIS and BARNES, 1963).

Work done many years ago by Dr. W. J. HAYES and his colleagues of the U.S. Public Health Service (DURHAM and WOLFE, 1962), demonstrated the degree to which spraymen were inevitably exposed during the application of DDT. It is impossible to apply any sprays of this type under conditions where the operator is given complete protection. However, under ordinary conditions the exposure is far greater by the dermal route from deposits falling on the skin than it is by inhalation.

Coupled with this knowledge is the experience already gained by the WHO Insecticide Testing Unit about the actual exposure to which spraymen and residents undergo when applying the newer types of organophosphorus (OP) or carbamate insecticides as residual indoor sprays (ELLIOTT and BARNES, 1963 - VANDEKAR, 1965).

Early work in Italy (MOSNA and ALESSANDRINI, 1954) had shown that spraymen applying diazinon indoors suffered falls in blood cholinesterase, but no symptoms, during a relatively short cycle of operations. When fenthion was first considered as a possible residual indoor spray, its acute single dose LD_{50} to rats being nearly twice as great as that of diazinon, it was believed that it, too, would be safe to apply. Indeed, it was safely applied when the operation was carried out with great care and with well protected spraymen. Used with less rigorous control, it rapidly produced severe poisoning among spraymen. It is probable that another factor comes into play, namely the very slow metabolism of this compound, so that the effects of a single dose appear after some delay and last several days in laboratory animals. Other observations made during spraying with carbamates also suggest that it may be possible to assess the likely safety of the compounds when sprayed on the basis of simple tests on laboratory animals.

The first tests on a new OP or carbamate insecticide are to determine the single oral dose LD_{50} to rats. As far as possible the nature and quantities of solvents used are kept constant. The time of onset, nature and duration of signs of poisoning are observed with particular attention to the duration of the effects of a single dose. The size of the LD_{50} alone may be enough information on which to say that a compound is likely to be

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too dangerous for conventional spraying. However, this will not necessarily preclude its use under conditions where no significant exposure of the operator will occur, as in some larviciding operations or in special devices as with dichlorvos.

If the single dose oral LD_{50} to rats is such as to suggest that the compound might be safe to apply, further tests are done. The first is to determine the single dose LD_{50} by skin application to rats. Compounds vary considerably in the ease which they penetrate the skin, and as this is the main route of exposure a compound that is almost as toxic by the dermal as by the oral route is likely to be particularly dangerous. The standard procedure is to apply the compound as a solution in an inert solvent such as dimethylphthalate after clipping the hair from the back of the rat. The rat is held in a wire cage so that it cannot lick the area, and after six hours this is cleaned with detergent and water. In a more recent technique, the treated area is covered with an impermeable dressing, and the rats allowed to move about freely. In most cases, the less toxic OP and carbamate insecticides have no measurable single dose LD_{50} by the dermal route as sufficient product cannot be put on the skin at a single application.

If a compound has a low dermal toxicity, the sensitivity of other species — mice, guinea pigs and rabbits — is compared to that of rats. Big variations in species sensitivity mean that care must be taken when people are first exposed to such a compound in case man is unduly sensitive. Repeated dosing by mouth and by skin application is then done over a short period to see whether the compound has unusual cumulative effects.

With OP compounds the therapeutic value of treatment by oximes is assessed.

In considering the likely safety of a new OP compound, features of its chemical structure are taken into account. Dimethylphosphates produce a more rapidly reversible inhibition of cholinesterase in acute doses than do diethylphosphates, and have proved consistently safer to use. On the other hand, diethylphosphates produce an inhibited cholinesterase that is more readily reversed by oximes so that treatment may be effective if the drug is available.

Another toxic effect of OP compounds for which watch is kept is the capacity to produce a delayed neurotoxic damage such as was seen in people poisoned with mipafox. It is now known that quite a number of different types of OP compounds can produce this delayed neurotoxicity in hens (ALDRIDGE and BARNES, 1966). Several compounds submitted to WHO for evaluation have been shown to possess this capacity, and recommendations made that no further work with them should be done. Thus, all OP compounds of novel structure are tested on hens. This may provide useful practical information as birds are sometimes much more sensitive than mammals to certain OP compounds. Domestic poultry may be exposed during house spraying operations, so that information on their sensitivity to a new compound may be useful.

The carbamate insecticides so far tested have all been monomethyl-carbamates producing the same type of inhibited enzyme. Oximes are of no value in speeding the reversal of this inhibition by carbamates. However, the different carbamates do exhibit striking variations in the relative affinity they show for the red cell and plasma cholinesterase.

From the work done in the Toxicology Laboratory of the Institute of Medical Research in Zagreb (VANDEKAR *and al.*, 1965), it is known that signs of poisoning are produced by a much smaller fraction of the LD_{50} of a carbamate than is the case with the OP compounds. In any animal tests special watch is needed to observe the dose at which signs of poisoning are first seen.

These relatively simple animal tests are considered to provide an adequate basis to make a decision as to whether a new insecticide, assuming it meets other criteria, may be applied as an indoor spray in further trials carried out by WHO. It is now recognized that all such trials will be done under the supervision of a toxicologist with facilities for making observations on the exposure of the spraymen. Thus, the operation could be suspended if there was any evidence that such exposure might be severe enough to lead to symptoms of poisoning.

Thus, a limited amount of toxicity testing on animals is all that is necessary when examining compounds with a well recognized mode of action as poisons, and to which people will only be first exposed to them under conditions where expert medical surveillance is available.

When a compound is submitted as an insecticide which does not fall into the groups of anticholinesterases or chlorinated hydrocarbon types, much more extensive tests will be needed, in order to assess its likely safety. In one such example the compound had a basic toxic action like that of an incoupler of oxidative phosphorylation (eg. like dinitroorthocresol). This made it seems improbable that it could ever be safely used in hot climates, but further tests showed that under some conditions it could produce limited but irreversible structural damage to the brain. On this basis it was considered quite unsafe for further testing as an insecticide.

At the present time there seems to be few compounds of novel structure and novel toxic activity on mammals which show promise as insecticides against the vectors of human disease. It is fortunate that so many different OP compounds and carbamates are available, and that so much useful experience of the hazards they present has now been acquired.

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