Relay toxicity. General principles of a new methodological approach for the toxicological evaluation of animal feed additives

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Original title: La toxicité de relais. Principes généraux d'une approche méthodologique nouvelle pour l'évaluation toxicologique des additifs aux aliments des animaux d'élevage


Translated by the Translation Section Department of the Environment

Department of the Environment Fisheries and Marine Service

Halifax Laboratory

Halifax, N.S.

1976

9 pages typescript
With the development of modern agricultural and food technology, an increasing number of chemical substances can be found in food destined for human consumption. This poses a fundamental problem for the toxicologist, who must demonstrate that the repeated absorption of actually very small doses of these substances through food intake every day, for a number of years or even a lifetime, does not produce any harmful effects, particularly over the long term. Numerous examples show that some of these compounds may build up in the system until concentration levels in susceptible tissues are such as to cause toxic effects, in some cases serious ones. For others, particularly those with the dreaded carcinogenic potential, the possibility of summation of the effects of each dose must be taken into consideration. Whatever the value of recent discoveries with respect especially to the possibility of repair to nucleic lesions and the existence of immunological defence mechanisms, the problem remains one of great concern (1, 2, 3, 4). It is essential not to authorize the use of chemicals which may later appear in foodstuffs until sufficient toxicological data have been obtained. There must be a guarantee at least that there is a very high probability of a chemical's being harmless even when it is repeatedly absorbed for virtually a whole lifetime. The problems of toxicological evaluation are different, of course,

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depending on whether the chemical is an intentional additive or an unintentional one. The former type is added deliberately and directly to human food, while the latter becomes incorporated in foodstuffs through a combination of circumstances, in particular through crops and animals that have been treated with the chemical prior to harvest and slaughter respectively. Among unintentional additives are pesticides, used to control crop pests, and animal feed additives used to stimulate growth and increase productivity. Aside from the bacteriological aspects, which are sometimes of great concern, the problem here for the food toxicologist is to ensure that the residue in foodstuffs obtained from treated crops or animals is not present in amounts or forms that would make it harmful to human health, especially when the foodstuff is consumed repeatedly on a daily basis.

The passage of a chemical substance through a plant or animal organism can have a whole series of sometimes unforeseeable consequences, some of which are harmful and some, beneficial.

In some cases, although there is no particular tendency toward accumulation, differences in the susceptibility of different animal species to toxic potential can come into play. The quail, for instance, can eat the fruit of the hemlock (Conium maculatum) without ill effect because of the bird's tolerance for cicutine, an alkaloid found in the plant. Quail which have eaten hemlock berries are toxic to man. Galliforms and frogs can also become toxic to humans by eating coleoptera containing cantharidin. Certain warm-sea fishes which cause ciguatera-type poisoning in man clearly appear to acquire their toxicity from the plankton they consume.

In other cases more directly related to the short- and long-term risks we have mentioned, foodstuffs and feed can become toxic as a result of the gradual concentration of substances with cumulative properties.

This possibility explains the interest of studying the passage through the food chain of environmental chemical pollutants. Build-up can occur with halogenated aromatic compounds, such as the DDT-type insecticides, hexachlorobenzene, polychlorinated biphenyls, and polychlorodioxines. It is also likely to occur with arsenic and the heavy
metals - lead, cadmium and mercury, in particular. A spectacular example of this is the acquisition of toxic properties by ordinarily edible fish and shellfish when mercury or its compounds, used for industrial or agricultural purposes, is discharged into fresh or salt water. These effluents are transformed into methylmercury compounds by the action of water-inhabiting bacteria and, according to some recent results (6), by enzymes found in the livers of certain species of fish. The compounds are very stable, highly neuro-toxic substances, able to pass through the placenta. The stories of the Japanese bays of Minimata and Nilgata, where whole communities were affected by mercury poisoning, are so well known that it is not necessary to recount them at length. We might, however, mention the observations recently published by Curley et al (7) concerning serious toxic effects in humans as a result of the consumption of pork from pigs which had been fed grain treated with organo-mercurial fungicides.

Conditions are not always so unfavourable. Often chemical agents applied to or introduced into plant or animal organisms are not absorbed or if they are, they disappear or are eliminated quickly. All that need be done is to determine, through appropriate analytical methods, the time necessary for their elimination. Harvest and slaughter conditions can then be set so as to rule out all risk to human health. However, the problems are more complex than it may appear at first sight. A chemical substance which enters a living organism rarely remains in its initial state. When it comes in contact with the many enzyme systems of the cells and the tissues, it is most often broken down into new products, called metabolites, the nature of which may vary according to the plant or animal species under consideration. Metabolites frequently represent forms of detoxification. To complement the example of mercury, mentioned above, it might be useful to mention seawater arsenic. When this element passes through the bodies of some crustaceans, where it builds up to high concentrations, it undergoes detoxifying changes. This happens, for example, in shrimps, whose relatively high arsenic content has been recognized since the analyses of Cox (8), Chapman et al (9), White (10) and Cheftel et al (11), among others. Through the prolonged administration to rats of diets made rich in arsenic by the addition of either arsenious oxide
or a quantity of shrimp containing an equivalent amount of arsenic, Coulson and his colleagues (12) clearly showed that, unlike the arsenious oxide, the arsenic in the shrimps showed almost no tendency to accumulate and cause long-term toxic effects. We can conclude from this that arsenic taken in from the marine environment by the shrimps is metabolized into relatively nontoxic compounds. During their passage through the rat's digestive tract, these compounds liberate soluble arsenic compounds, which are rapidly eliminated by way of the kidneys.

Unfortunately many other examples besides that of methylmercury, which has already been mentioned, prove that the passage of chemical compounds of exogenous origin through living organisms may equally result in the formation of toxic metabolites. Some of these show a tendency to build up, constituting a serious health hazard for those who eat the organism.

Sometimes, in the case of animal feed additives, enzymes produced by the bacteria of the intestinal flora transform the ingested compound into metabolites, which, far from always being forms of detoxification, can be much more toxic than the original additive. Cyclamates, nordihydroguaiaretic acid and chocolate brown FK are classic examples of this.

Aware of this fact, toxicologists have demanded the testing of the metabolites found upon analysis, when pesticides or animal feed additives are used. In spite of the undeniable value of this approach, many difficulties remain. For one thing, numerous metabolites, not all of which are necessarily easy to identify, can be formed from a given compound. The general tendency is to place the greatest emphasis on the toxicological evaluation of the metabolite or metabolites formed in the greatest quantity. However we cannot dismiss a priori the possibility that a metabolite produced in a minor proportion may toxicologically be very significant. Neither can we dismiss the possibility that unforeseeable effects may result from interactions between the various residue substances or between a residue substance and one of the other chemical substances which modern man is constantly apt to absorb. There are other possibilities. Enzyme induction may cause derivatives of certain endogenous compounds,
in particular the hormonal steroids, to build up to abnormally high levels. The derivatives, either through loss of the original properties of their precursors or through acquisition of new properties, are liable to upset homeostasis, thus disturbing the physiological functions. Then there is the possibility of reaction with certain constituents of body tissue, particularly proteins. This process could constitute a form of stockpiling; toxic substances, concealed in the reaction products, could later be released into the digestive tract. It is clear therefore that the toxicologist, in consideration of the facts above concerning the passage of a chemical substance through a plant or animal relay, must alter his approach if he is to evaluate properly hazards to human health. He must evaluate not only the initial substance (as in the case of intentional additives), the nature and quantity of which are perfectly known, but also the quantitative and qualitative characteristics of all the residues resulting from the relay phenomenon.

The classical approach to the toxicological evaluation of these residues - subjecting the substance and its main metabolites to acute, short-term, semichronic and long-term toxicity tests - would be a most tedious job. Moreover, it might lead to false security because of the inevitable uncertainty factor when the results are interpreted, and especially when they are extrapolated to man. This is why we think that the foodstuff itself, in all its complexity, should be evaluated.

Rationally speaking, this approach is obviously much more satisfactory than carrying out toxicological tests on laboratory animals, as in the case of human food additives. The test substance may not be absorbed by the farm animal or, when passing through the animal's system (relay) it may disappear or be transformed by metabolic processes completely different from those occurring in the laboratory animals. It would then, be acceptable to simplify the toxicological studies carried out before the effectiveness of the additive in livestock feed is evaluated. Only the information necessary to protect the health of the farm animals would need be obtained. More detailed toxicological studies
carried out at this stage would be of only limited value for the human consumer, because the passage of the substance through the animal relay can put the validity of the findings in doubt. In any case, finding out what happens to the additive on its journey is very important in deciding what approach to take.

Unfortunately, there is a major difficulty in the methodology of toxicological evaluation, in that a large safety factor must always be allowed if results are to be extrapolated from the animals to man. A factor of 100 is commonly used in evaluating intentional additives. While recognizing that this value is not unalterable and that consequently experts, using common sense and scientific judgement may logically advise choosing a lower one, it is none the less clearly impossible, for nearly all foods, to administer to laboratory animals a daily dose by unit body weight that is far above the average dose consumed by man.

Even if a high safety factor can be achieved in an experimental diet, there is very often a risk of producing serious disturbances in the test animal, either because of its inability to accept the food or, more likely, because of nutritional imbalances. To understand this one need only think of the effects which would be produced in a laboratory animal by the administration of doses of sucrose or sodium chloride (expressed in milligrams per kilogram of body weight) 100 times superior to those normally consumed by man. It is also known that normal human diets administered to rats or dogs for prolonged periods may sometimes generate pathological disturbances in these animals (13, 14). There is, then, great value in ensuring that the diets administered to test animals meet their nutritional needs. It is true that for traditional foods, data accumulated from observing man himself represent the best safety factor. The difficulty remains when the foodstuff, even if it is a very ordinary one, contains residues of which toxicological evaluation has not yet been made. Food exposed to radiation, for example, may contain products which were not initially present and of which it is often very difficult to make a complete quantitative and qualitative inventory even by use of the most minute analysis. One approach would be to concentrate the substances to be evaluated in fractions but long, laborious research would be necessary.
before this method could have practical application. At present, all we can achieve is a rational approximation, which will always be subject to revision. As more is learned on the nature and quantities of the products formed under the action of radiation in strictly defined conditions, this approximation will be refined.

Is this to say that we can never, merely by experimenting with foods which may contain residues, contribute to their toxicological evaluation? Experience inclines us toward the opposite opinion. Firstly, if the results of such experiments show grounds for rejection, they may spare us long and expensive testing. If noxious effects become apparent when the food suspected of containing residues is administered to test animals as part of their diets, without any safety factor being applied, adequate proof exists that the residues pose a hazard. Use of the tested product should not be authorized. An excellent illustration of this is that of diethylstilbestrol, which, when implanted in calves, gives their meat properties that inhibit the reproductive function. However when an equivalent quantity of diethylstilbestrol, calculated according to the implant dose, is added to the same meat, no inhibitive properties are apparent. This suggests that complex reactions, which we as yet know nothing about, occur during the passage of the synthetic estrogen through the relay system.

Conversely, can the results obtained from comprehensive food testing constitute a criterion of acceptability? To this we would answer in the affirmative in so far as certain animal feed additives are concerned. The essential conditions are that food of animal origin must constitute only a limited percentage of average human food intake. Also, it must be possible, without provoking nutritional imbalances, to increase this percentage considerably in the diets of laboratory test animals; control animals should receive a diet containing the same percentage of the same food obtained from farm animals whose feed did not contain the additive. It is possible to increase the safety factor by increasing the concentration of the additive in the diet of the relay animal. Furthermore, the safety factor is considerably increased because of requirements concerning the use of animal feed additives. They nearly always call for suspension of treatment before slaughter,
sometimes for long periods, which means that the amount of residue gradually decreases in most cases.

In an article presently in press, we shall describe the application of our new methodological approach in the toxicological evaluation of a non-antibiotic growth factor recommended for pigs. We hope our results will show that the growth factor may be considered acceptable, at least provisionally, even though the additive proved carcinogenic when it was tested directly by administering large doses to rats. On this basis, authorization of its use would have been refused.

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