

'COCKTAIL' EFFECTS – STIRRED, NOT SHAKEN....YET

Stewart Reynolds and Alan Hill from the Central Science Laboratory, York, UK, discuss the controversy over the possibility of greater risks associated with multiple pesticide residues, popularly called pesticide 'cocktails'

Introduction

Although pesticide residues are frequently found in many of the foods that we consume on a daily basis, most of them occur at extremely low levels and, on the basis of the best available scientific knowledge, pose no measurable threat to the health of consumers (Bates, 2002). However, the best available scientific knowledge is almost entirely composed of toxicological data produced from doses of the pesticides given singly, not in combination.

In some commodities only single pesticides are detected, but in others, more than one pesticide, may be present. Where two or more pesticides occur in combination, they are known as multiple residues or, colloquially, as residue cocktails. Up to 11 different pesticide residues have been detected in retail samples taken and analysed as part of the UK's annual national monitoring programme (WPPR, 1997; WPPR, 1998; WPPR, 1999; PRC, 2000). These multiple residues are often combinations of fungicides, insecticides, growth regulators, herbicides and antioxidants and they can be found in both UK-produced and imported commodities. Conventionally grown crops, such as apples, celery, citrus fruit, grapes, lettuce, peaches/nectarines, potatoes and strawberries, are all likely to be treated with various pesticides, for different purposes and at different stages of crop production, which will give rise to multiple residues in the resultant retail produce.

It must be acknowledged that the numbers of pesticides found in combination, and the numbers of samples in which combinations of pesticides are found, are probably underestimated. This is because there are practical and economic limits to the range of pesticides which can be detected by analysis and to the sensitivity of detection. Indeed, were it not for the major advances in analytical technology that have occurred in recent decades, most of the multiple residues presently detected would have remained unobserved.

However, it must also be acknowledged that some apparent multiple residues in fruit, vegetables or cereals are not multiple residues at all. They appear to be multiple residues simply because of mixing of produce, during grading, packing, *etc.*, which has been subjected to different treatments. Similarly, manufactured multi-component foods may contain multiple residues simply as a consequence of mixing products that contain only single pesticides. Exposure to a 'cocktail' of residues could also arise from consumption of several foods each of which contains only single residues. In this respect, preparing a meal is similar to the manufacture of multi-component foods.

For the purposes of distinguishing the various scenarios, we will refer to:

- *Primary 'cocktails'* – 'cocktails' derived from treatment of a single commodity with multiple pesticides
- *Secondary 'cocktails'* – 'cocktails' derived from mixing several commodities (*e.g.* a mixed fresh fruit salad or a multi-component meal)

Origins of primary 'cocktails'

Man is not alone in needing food. Foodstuffs at all stages of production, transport and storage, and food producing areas, represent excellent opportunities for the growth and multiplication of a host of pests and diseases; many of which, if not adequately controlled, are capable of quickly producing serious shortages of food. Pesticides form only one, albeit important, of the many strategies required for producing sufficient food, but the multitude of pests and diseases to be combated requires pesticides with different chemical modes of action. In many cases, pests and diseases have the capacity to develop resistance if a single pesticide is used repeatedly and, in some cases, the pest or crop requires different pesticides at different growth stages for adequate control. It follows that there is a need to use a range of pesticides in the production of any one food, sometimes resulting in primary 'cocktails' of residues. In general, the more persistent the pesticide and/or the more active ingredient required for control, and/or the later it is used in the production/storage cycle of the foodstuff, the more likely it is that residues will be detectable. Inevitably, primary and secondary 'cocktails' tend to be comprised of pesticides with these characteristics. We will illustrate this with a few examples:

Potatoes

Potatoes are a very important part of the diet in many countries, producing relatively large quantities of food per unit area of land. But potatoes are subject to attack from several potentially devastating pests and diseases and they do not crop well in competition with weeds. Pesticides applied to potatoes before harvest rarely give rise to measurable residues, with the exception of maleic hydrazide, which is partly intended to control sprouting of tubers after harvest. Sprouting suppressants, such as chlorpropham or maleic hydrazide, and fungicides such as thiabendazole or imazalil, which are applied post-harvest are frequently found as residues.

Citrus fruit

Citrus fruit (oranges, lemons, grapefruit, satsumas, *etc.*) are frequently associated with primary 'cocktails' of residues but, unlike potatoes, some of these arise from treatments made during the long growing season. This is particularly true of insecticides such as chlorpyrifos and parathion-methyl and acaricides such as bromopropylate. Unusually, citrus fruit also often contain residues of the growth regulator (and herbicide) 2,4-D, which is applied to prevent premature abscission at the fruit (which would lead either to premature fruit fall or loss of the stems post-harvest, allowing the ingress of fungi which cause rotting); this pesticide, therefore, frequently occurs in primary 'cocktails'. After harvest, citrus fruit may be held in transit or storage for some time and, during this period they are prone to attack by a range of fungi and effective control may only be achieved by using a combination of fungicides, such as imazalil, thiabendazole and/or 2-phenylphenol; these pesticides also occur frequently in primary 'cocktails'.

Strawberries

Strawberries, like potatoes, are very susceptible to attack from a number of insect, mite and nematode pests, several devastating fungal diseases, and they do not compete well with weeds. Pesticides applied pre-planting, at planting, or pre-blossom, rarely give rise to detectable residues in the fruit. Later in the growing season, strawberry plants and fruit are very prone to attack by fungi, such as botrytis and powdery mildew. These diseases are often treated with a number of different fungicides, not only to inhibit development of the various fungi, but to avoid the possible development of disease resistance to any particular chemical. These pesticides are frequently detectable at harvest and therefore frequently occur as primary 'cocktails'.

Primary 'cocktails' in UK surveys

Table 1 shows some of the multiple residues detected in a PRC survey of strawberries purchased from a selection of retail outlets in the UK during 2001 (PRC, 2001). Strawberries are too fragile to be mixed post-harvest and each punnet in each sample was marked with a single grower's code, so it is reasonable to assume that each sample was derived from a single crop. All the residues were of fungicides, and these 8 samples from 8 different countries were chosen specifically to illustrate the wide range of fungicides (16 different compounds) which were used and resulted in measurable residues. Some of these fungicides are from the same class of compound, for example, azoxystrobin, kresoxim-methyl and trifloxystrobin are strobilurins that have the same mode of action, inhibiting fungal spore germination and mycelial growth.

Probability of the occurrence of primary 'cocktails'

The residues data from the UK national monitoring programme can also be used to identify the probabilities with which specific pesticides have occurred in combination with other pesticides in various foodstuffs. Table 2 shows

Table 1. Multiple residues detected in retail samples of strawberries obtained during 2001

Sample	Pesticide	Residue level (mg kg ⁻¹)	Country of origin
1	bupirimate	0.09	UK
	fenhexamid	0.6	
	iprodione	0.6	
	pyrimethanil	0.05	
	tolyfluanid	0.1	
2	captan	0.7	The Netherlands
	fenhexamid	0.08	
	penconazole	0.1	
	tolyfluanid	0.2	
3	captan	0.2	Egypt
	chlorothalonil	0.5	
	iprodione	0.3	
4	kresoxim-methyl	0.03	Israel
	penconazole	0.2	
	trifloxystrobin	0.06	
5	azoxystrobin	0.03	Portugal
	bupirimate	0.03	
	captan	0.03	
	cyprodinil	0.1	
	fenhexamid	0.2	
	procymidone	0.04	
6	metalaxyl	0.06	Spain
	procymidone	0.04	
7	captan	0.1	Egypt
	carbendazim	0.1	
8	fenhexamid	0.2	USA
	pyrimethanil	0.2	

the worst-case frequencies of occurrence of certain pesticides in multiple residues in apples surveyed over a 4-year period. Worst-case frequencies are recorded to avoid problems of interpretation associated with different pesticides being sought, and different reporting limits being adopted, for different surveys.

Table 2 has been assembled from five surveys of apples performed during the period of January 1997 to December 2000. Of the pesticides listed, carbendazim (probably), thiabendazole (probably) and diphenylamine would have been applied post-harvest. The remainder would have been applied post-blossoming. The data in Table 2 represent 'cocktails' of 2–5 pesticide residues in a total of 85 different combinations. The probabilities of occurrence with which multiple residues of various combinations are predicted from the data in Table 2 are similar to the frequencies with which the cocktails occurred in practice. So, for example, Table 2 predicts that the probability of co-occurrence of carbendazim and diphenylamine in apples would be 15.2% of samples (39% × 39%). Surveys which included these two

Table 2. Pesticides ranked by frequency (% of samples) of worst-case occurrence of multiple residues in apples monitored during the period 1997-2000

Pesticide	%	Pesticide	%
carbendazim	39	bifenthrin	4
diphenylamine	39	bromopropylate	4
chlorpyrifos	25	ethoxyquin	4
thiabendazole	17	iprodione	4
dithiocarbamates	14	dithianon	3
metalaxyl	14	bupirimate	3
captan	10	myclobutanil	3
phosalone	8	diazinon	1
propargite	8	paclobutrazole	1
carbaryl	8	tolylfluand	1
dimethoate	8	omethoate	1
pirimicarb	8	dicofol	1
dodine	6	methomyl	1
azinphos-methyl	4	tebufenpyrad	1

pesticides were conducted in 1997 and 1998 and the actual frequencies of co-occurrence were 16.7 and 12.8%, respectively (average 14.7%).

Probability of occurrence of secondary 'cocktails'

Although residues data from manufactured foods might be considered a suitable source of information on secondary 'cocktails', when these products are analysed it is impossible to distinguish between primary and secondary 'cocktails'. However, using the frequencies with which pesticides have occurred (singly and in 'cocktails') in a wide range of foodstuffs surveyed in the UK, together with UK dietary data, it is possible to estimate the probability with which an average or extreme consumer takes in a measurable quantity of each pesticide on a daily basis. Table 3 provides an illustration of these data. The probability with which both primary and secondary 'cocktails' occur in the diet can then be estimated by multiplying the probabilities of occurrence of the individual pesticides.

Hazard, exposure and risk assessment

Hazard

What is the combined effect of 'cocktails' (acute and/or chronic) to humans? Is it purely additive, or do additional synergistic or antagonistic effects have to be taken into account when performing risk assessments? Some pesticides have similar toxic actions, whilst others have very different modes of action. The genotoxicity of benomyl and pirimiphos-methyl was studied separately and combination (Piatti *et al.*, 1994) and no interaction was found between the two pesticides which have very different toxic actions. Pesticides having the same end-point (effect) do not necessarily have the same mode of action at the biochemical level.

Under the Food Quality Protection Act (FQPA), 1996, the US Environmental Protection Agency is charged with

Table 3. Ranking of the relative probability of occurrence of the top 20 pesticides in the adult diet estimated from chronic intake of foodstuffs

Rank	Pesticide	Relative probability
1	DDT	47
2	thiabendazole	43
3	imazalil	39
4	gamma-HCH	36
5	dieldrin	27
6	HCB	27
7	carbendazim	25
8	iprodione	19
9	2,4-D	18
10	chlormequat	17
11	inorganic bromide	17
12	dithiocarbamates	17
13	2-phenylphenol	15
14	chlorpyrifos	15
15	chlorpropham	12
16	oxadixyl	11
17	methidathion	11
18	maleic hydrazide	10
19	beta-HCH	9
20	diphenylamine	9

making cumulative risk assessments. In such assessments, the EPA must take into account the combined effects of different pesticides having a common mode of action (EPA, 2002, Wilkinson *et al.*, 2000). The assessments are very difficult to make in the absence of definitive data and there are only a few groups of pesticides with a well-defined, common mode of action. For example, the organophosphorus and carbamate pesticides produce adverse effects similarly, by inhibiting acetylcholinesterase activity. In addition to the questions regarding possible synergism, there is the question of whether certain groups of the population, such as pregnant women and infants, may be at higher risk of 'cocktail' effects than the general population. Again, all data on reproductive and developmental toxicity of pesticides has been generated from single compounds, so there is virtually no evidence regarding the effects of 'cocktails' at low doses, *i.e.* the levels at which pesticide residues are found in food.

Exposure

Before considering the toxicology of 'cocktails' further, it is as well to consider issues that affect the actual intake of 'cocktails'. That is, if a consumer eats a product containing a 'cocktail' of residues, estimation of the intake of pesticides involved may appear simple but it is not necessarily so. Most residues data on primary foodstuffs are generated from samples that have not been prepared for eating, by peeling, chopping, cooking, *etc.* In these processes, some pesticides are partly or wholly lost through volatilisation, degradation or mechanical removal and therefore the intake of residues may be much lower than predicted from the original residue concentrations. These so-called processing

factors are important for making realistic assessments of risk, whether for single residues or for 'cocktails'.

It is inappropriate to assess the frequency with which residues occur in UK foods from survey data. The problem is that UK surveys are small, and most are targeted at produce where residues are more likely to occur, and a large-scale random programme of surveillance would be necessary to assess the frequency of residues, including multiple residues. Furthermore, data on exposure from sources other than food and water are extremely limited or non-existent.

Risk assessment

Before dismissing data on the toxicology of single pesticides, it would be well to recall that risk assessment indices (such as the acceptable daily intake, ADI, and the acute reference dose, acute RfD) generally incorporate a factor of 10 margin for inter-species differences and another factor of 10 for intra-species differences. So generally a safety margin of a factor of 100 is applied. In addition, in the admittedly few cases in which there are data available, humans have proven less susceptible (*i.e.* they are not 10 times more susceptible) than the test species on which the indices are based. Whilst the thalidomide incident is a good reminder that we should not be complacent, it is clear that the 'cocktail' effects would have to be dramatically more adverse than those of single pesticides if current safety limits are to be found inadequate.

The health risks of residue 'cocktails' have been considered in two recent reviews:

European Crop Protection Association review

In 2000, the European Crop Protection Association (ECPA) published a critical review (Carpy *et al.*, 2000) on the health risks posed by low-dose pesticide mixtures. This literature review covered a fourteen-year period between 1985 and 1998 and concentrated on combination toxicology and human risk assessment relating to exposure to the levels of pesticides that occur in the human diet and environment. It was concluded that there could be no single methodological approach to 'combination toxicology' and health risk assessment of chemical mixtures. Also, data demonstrated that exposure to a combination of chemicals does not cause stronger effects than that of the most active component, provided that the components are at low concentration levels, *i.e.* below their respective no-observed-adverse-effect levels (NOAELs). A combination of chemicals with the same target organ and the same, or very similar, mechanisms of action may cause additive or even synergistic effects. The chances of such an effect diminishes as exposure levels decrease. Synergism and antagonism may both occur at the same time at different organs or targets in the same organism. However, interaction between components is not common at the low levels of human exposure as those that may occur from pesticide residues in food and drinking water. Thus the routine use of an additional safety factor for mixtures in addition to those normally employed for deriving acceptable daily intakes (ADIs), acute reference doses (acute RfDs), or minimal risk levels, is not supported by data.

The review concluded that, as a general rule, exposure to mixtures of pesticides at low doses of the individual

constituents does not represent a potential source of concern to human health.

UK Committee on Toxicology WiGRAMP Report

At about the time that the ECPA published its review, the UK Food Standards Agency (FSA) and the UK Committee on Toxicology of Chemicals in Food, Consumers Products and the Environment (COT) set up a working group to review critically the analytical and toxicological data on mixtures of pesticides and similar, and to consider the implications for the risk assessment process. In December 2000 an independent Working Group for the Risk Assessment of Mixtures of Pesticides and Veterinary Medicines (WiGRAMP) was asked to consider:

- whether there was any scientific basis for consumer concerns about the occurrence of multiple residues of pesticides and veterinary residues and the resulting 'cocktail' effect
- whether regulatory bodies should consider all pathways of exposure to pesticides (*e.g.* through food, water, professional and household uses, pet treatments) (also known as aggregate exposure)
- if it was considered appropriate to consider concurrent exposure to more than one pesticide, what assumptions could be made about the combined toxicity.

In 2002, the Committee on Toxicity published a comprehensive draft report (COT, 2002) in which many of the findings agree with those of the ECPA review.

The report suggests that in addition to deterministic (worst-case, fixed value) methods of risk assessment, probabilistic methods (Hamey, 2000), including intakes from all sources, should be considered for both aggregate and cumulative risk assessments. Further research was considered necessary, initially to establish the true probability of exposure of the UK population to residues in food. This will be difficult as current UK surveillance is targeted at produce where residues are most likely to occur, rather than using a random monitoring system to assess the frequency of occurrence of residues, including multiple residues. WiGRAMP recommend the use of biomarkers of exposure to provide more cost-effective methods for assessing exposure. The problem will be to identify suitable biomarkers that can provide reliable detection of systemic responses and potentially adverse health effects arising from exposure.

Finally the report recommends that research should be undertaken to characterise the dose-response relationship for the combined actions of pesticides, veterinary medicines and similar substances which have common targets of toxicological action. For a realistic assessment of 'cocktail' effects, the potential impact of non-pesticidal toxicants (natural and synthetic) to which consumers are exposed should not be ignored (Feron *et al.*, 1998 and Miller, 1997). However, taking all toxicants into account, countless 'cocktails' become possible and it is unlikely ever to be practical to identify and test all of them.

These reports confirm earlier conclusions drawn by the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO

Expert Group on Pesticide Residues (JMPR) (FAO/WHO, 1997). The JMPR also concluded that the safety factors that are used for establishing ADIs should provide a sufficient margin of safety to account for potential synergism.

Conclusions

The risk assessment of multiple pesticide residues is hampered by both a lack of data on the toxicological effects of mixtures of pesticides at very low levels and clear evidence of the actual exposure of consumers to such residues. Although various studies addressing this issue have been performed in the last 20 years, those that show non-additive (antagonistic or synergistic) effects have been performed at 'effect doses', which are not relevant to mixtures of pesticide residues that may be present in food at levels several-fold lower than effect levels.

For example, experiments demonstrating synergistic effects between the organochlorine pesticides chlordane, dieldrin, endosulfan and toxaphene in competition for human estrogen receptor (recombinant or transiently transfected into yeast cells) (Arnold *et al.*, 1996) showed that 'cocktails' of the pesticides were some hundreds of times more potent competitors of 17 β -estradiol than when tested separately. Competition did not increase to 100% at any concentration and EC₅₀ (effect concentration) or IC₅₀ (inhibition concentration) concentrations of the 'cocktails' were $\geq 0.1 \mu\text{M}$ (equivalent to about 0.04 mg/kg). It is, of course, difficult to relate these concentrations to those which might occur at the receptor *in vivo* but the highest levels found in animal fat (which contains by far the highest concentration of these fat-soluble chemicals) in UK surveys rarely exceed this concentration. Nonetheless, many other environmental contaminants and natural constituents of foods (*e.g.* phytoestrogens) could also interact – synergistically or antagonistically – with pesticide residues. However, unlike the few very stable organochlorines, such as chlordane, dieldrin and toxaphene, endosulfan and most other pesticides are not persistent in the body and therefore, in practice, even a proven effect of most 'cocktails' would require a coincidence of occurrence at the site of effect.

The assessment of risks from 'cocktails' of pesticide residues cannot be approached simplistically but there is little evidence to suggest that they pose any greater or lesser risks to consumers than the sum of individual residues. There is very little evidence to suggest that the vast majority of individual residues have any adverse effects on consumers, either, but it would be naive to think that this is the end of the story. The cocktail continues to be stirred, but has yet to be properly shaken. Future research will probably be undertaken on chemical mixtures in cellular and sub-cellular systems *in vitro*. Valuable as such data may be, it will not be possible to interpret the results properly without resorting to experiments on whole animals – with all the costs and ethical problems that that entails. The subject has the potential to keep toxicologists employed for decades to come but it may equally well become a career graveyard. That's the problem with cocktails, you cannot tell how potent they are until after you have tried them.

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