Session 4A

Pest and Disease Management in Potatoes

Chairman	Dr G Jellis
Session Organiser	Dr K Evans
Papers	4A-1 to 4A-4

PROSPECTS FOR CONTROLLING NEMATODE PESTS OF POTATO, PARTICULARLY POTATO CYST-NEMATODES

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ABSTRACT

The ten genera of nematodes that attack potato may be controlled by one or more of five control measures, i.e. crop rotation, resistant potato cultivars, trap cropping, soil solarization and nematicides. Potato golden cyst-nematode, *Globodera rostochiensis*, has been well controlled by combined use of rotations, resistant cultivars and nematicides. Potato pale cyst-nematode, *G. pallida*, is harder to control because there are very few agronomically acceptable cultivars with useful resistance to it and because granular nematicides effective against *G. rostochiensis* are generally less effective in controlling multiplication of *G. pallida*. Combined use of effective and partially effective control measures in appropriate integrated programmes is essential for the control of *G. pallida*. Measures which may improve control of potato cyst-nematodes in the future are outlined.

THE NEMATODE PESTS OF POTATO

Ten genera of nematodes feed and multiply on potato (Table 1). Apart from the stem nematode, *Ditylenchus dipsaci*, which attacks shoots and sometimes the tubers, it is the roots and often the tubers that are parasitized. The most important nematode pests of potato are cyst-nematodes, *Globodera* spp., reported from 48 countries (Evans & Stone, 1977), followed by root-knot, root-lesion, tuber-rot and stubby-root nematodes.

Potatoes are grown for three to eight months in ridges or raised beds and the fibrous root system is most developed in the top 40-50 cm of the soil, where most nematode parasites are found. Potatoes are damaged by nematodes (a) feeding on plant tissues, (b) interacting with and exacerbating other diseases, e.g. *Verticillium* wilts and (c) transmitting certain pathogenic viruses, e.g. some stubby-root nematodes can transmit tobacco rattle virus causing "spraing". Direct damage by cyst-nematodes, with only one full generation on a potato crop, is largely determined by the initial population density (Pi) to which the young root system is exposed. Cultivar, soil moisture regime and soil pH may also affect yield loss (e.g. Mulder, 1994). Damage by sting, needle and stubby-root nematodes, all of them root ectoparasites, also depends heavily on the duration of adequate soil moisture to allow the nematodes to move, aggregate and feed at the tips and along the sides of roots. Nematodes other than cyst nematodes complete two or more generations per season and the new generations usually contribute to damage. Damage is prevented when Pi is decreased to the damage threshold density (Pt). Even

greater decrease may be needed to prevent virus transmission.

TABLE 1. Nematode pests of potato

Common name	Latin name	Plant parts attacked
World-wide distribution		
golden cyst-nematode	*Globodera rostochiensis*	roots and tubers
pale cyst-nematode	*G. pallida*	" " "
root-knot nematodes	*Meloidogyne* spp.	" " "
root-lesion nematodes	*Pratylenchus* spp.	" " "
tuber-rot nematode	*Ditylenchus destructor*	tubers
stubby-root nematodes	*Trichodorus* spp. and *Paratrichodorus* spp.	roots
Limited distribution		
sting nematode	*Belonolaimus longicaudatus*	roots
needle nematodes	*Longidorus* spp.	"
stem nematode	*Ditylenchus dipsaci*	shoots and tubers
false root-knot nematode	*Nacobbus aberrans*	roots and tubers
reniform nematode	*Rotylenchulus reniformis*	roots

Unlike damage, which is *proportional* to population density, nematode multiplication is *inversely proportional* to population density. Thus, reducing Pi to Pt, thereby preventing crop damage, favours much multiplication by the surviving nematodes. Avoidance of any increase in nematode numbers will only be achieved when Pi is reduced sufficiently to counterbalance multiplication by survivors. Except for *Paratrichodorus minor* (syn. *Trichodorus christiei*), root-ectoparasitic nematodes increase slowly in a season (up to 10-fold), so they are controlled by reducing their numbers by about 90%. Cyst-nematodes can multiply 50-fold or more, so a reduction of 98% or more may be needed. Root-knot and other nematodes may increase 1000-fold, requiring a reduction of 99.9% in Pi. Population reductions in excess of 90% are not usually attained by any single method of control applied once.

METHODS OF CONTROL WITH SPECIAL REFERENCE TO CYST-NEMATODES

Currently, five control measures, singly or in various combinations (integrated control), may keep nematode numbers at or below Pt. All of these measures have been more or less effective against cyst-nematodes and all potato nematodes have been controlled to a greater or lesser extent by fumigant or non-fumigant nematicides (Table 2). Several control measures are ineffective, inappropriate or have not been assessed for

nematode pests other than cyst-nematodes.

TABLE 2. Control measures more or less effective (+), possible (+/-), ineffective or inappropriate (-), or awaiting study (?) against nematode pests of potato.

Nematode	Control measure					
	Rotation	Trap-cropping		Nematicides		
		Resistance		Solarization		Integrated
Cyst	+	+	+	+	+	+
Root-knot	+/-	?	?	+	+	+
Stem, tuber-rot	+/-	+	-	?	+	+
Root-lesion	-	?	-	+	+	?
False root-knot	+/-	?	?	?	+	?
Stubby-root	-	?	-	?	+	?
Sting	-	?	-	?	+	?
Needle	-	?	-	?	+	?
Reniform	-	+	?	?	+	?

Potato cyst-nematodes: distribution and abundance in soil

Potato cyst-nematodes are most abundant in the top 40-50 cm of the soil but, at half the sites examined in a survey of heavily infested soils in England, the top 20 cm was more heavily infested than soil 20-40 cm deep (Whitehead, 1977). When susceptible potatoes are grown, very small infestations may increase up to 150-fold, though increases of more than 50-fold are uncommon in commercial practice. Nevertheless, infestations of up to 3,000 eggs g^{-1} of soil have been found.

Crop rotation

Most nematodes that attack potatoes have wide host ranges, making control by rotation of crops either difficult or, if the non-host crops are unmarketable, impractical. The effectiveness of control by rotation of crops is further lessened by weed hosts and self-sets of susceptible crops. In contrast, potato cyst-nematodes only multiply on certain Solanaceae, notably potato, tomato and aubergine. In Britain they increase little or not at all on solanaceous weeds (Whitehead, 1985). They can, therefore, be controlled, at least partially, by rotations in which non-solanaceous crops predominate. Estimates of the resultant decline in numbers of eggs within the cysts under non-host crops have varied from 20-49% per annum. In Sweden, faster rates of decline have been reported in the first year after potatoes and in subsequent years in N. Sweden (Andersson, 1987). When soil infestations are large and decline rates are slow, very long rotations are needed to decrease population densities sufficiently to prevent damage (Table 3). In microplots of different soils without volunteer potatoes, three populations of *G. rostochiensis* and five of *G. pallida* declined at rates of 13-25% per annum under barley. A fourth population of *G. rostochiensis* declined faster (41% per annum) (Whitehead,

1995 (in press)). Under oats, at population densities less than the equilibrium density, four volunteer potatoes m^{-2} significantly increased potato cyst-nematode population densities (den Ouden, 1967). In widely spaced row crops, tractor-hoeing may lessen this effect and destroy volunteers from potato seed but would have little impact on volunteers from deeply buried tubers. Provided the tuber or tuber piece has adequate reserves, the nematode can complete its life cycle on the roots of underground shoots. Killing volunteers before the life cycle is completed may hasten nematode decline. Spraying volunteers in the first three weeks of June with glyphosate has reduced numbers of viable eggs of both species of *Globodera* (Jellema & Brinkman, 1986).

TABLE 3. Years rest from potatoes for decline of potato cyst-nematode to 5 eggs g^{-1} soil.

Decline (%) per annum	Eggs g^{-1} soil		
	50	200	800
50	3.3	5.3	7.3
30	6.5	10.3	14.2
20	10.3	16.5	22.7

Resistance, susceptibility and tolerance

Cultivars resistant to reniform, tuber-rot and cyst-nematodes have been bred. No cultivar is, or is likely to be, resistant to all potato nematodes, so exotic species, pathotypes or races should be strictly quarantined. For example, the potato race of false root-knot nematode, *Nacobbus aberrans*, a serious pest of cool soils in S. America and Mexico, is unknown in Europe and must be kept out. South American populations of potato cyst-nematode are genetically more diverse than European populations and are able to overcome resistance conferred by genes currently incorporated in resistant cultivars in Europe (Franco & Evans, 1978).

A fully resistant cultivar prevents multiplication of invading nematodes so soil populations decline markedly. A cultivar fully resistant to *G. rostochiensis* can decrease soil infestation by 80% in a season, so that, in theory, three consecutive years of growing it should decrease infestation by more than 99%. Such a reduction was reported by Zawiślak *et al.* (1981), following four years of a resistant potato. Even partially resistant cultivars are useful, because they permit less multiplication than most susceptible cultivars.

Over the last fifteen years many cultivars (at least 123) have been available with resistance (nearly always full resistance) to *G. rostochiensis* pathotypes Ro1 and Ro4. A few cultivars were bred with resistance to other pathotypes, e.g. Esta and Heidrum (Ro1 and Ro5), Pansta (Ro1-Ro4) and Miranda and Franzi (Ro1-Ro5). Thirteen cultivars were bred with partial resistance to Pa2/3 and, in Peru, cv. Maria Huanca is resistant to P5A (Pa3) and partially resistant to P4A (Pa2) but susceptible to P6A (no equivalent

pathotype in Europe). Where crop quality is less important than yield, as in potato starch production and subsistence farming, the grower has a wider choice of resistant cultivar than the grower of potatoes for pre-packing or processing where quality is paramount. In the 1995 British NIAB list of recommended and provisionally recommended cultivars, five of the nine first early and nine of the twenty second early and main-crop potatoes are resistant to *G. rostochiensis* Ro1 and Ro4 and second early cv. Nadine and main-crop cv. Santé are also partially resistant to *G. pallida* Pa2/3. Although the widespread and often frequent cultivation of potatoes resistant to *G. rostochiensis* but susceptible to *G. pallida* - notably cv. Maris Piper - has greatly decreased the former in southern England, it has greatly increased the latter, so that *G. pallida* is now the dominant species throughout the traditional ware potato areas of England. Continued, widespread use of cv. Maris Piper and other cultivars with the same narrow resistance will intensify selection of *G. pallida* and may reveal hitherto undetected Ro pathotypes, as occurred in an experiment by Øydvin (1978). Similarly, cultivars partially resistant to *G. pallida* may select virulent strains from field populations (Turner, 1990; Whitehead, 1991).

Nematode multiplication can vary appreciably between susceptible cultivars. On cv. King Edward, *G. rostochiensis* increased twice as much as on five other cultivars (Whitehead *et al.*, 1980) and *G. pallida* multiplied much more on cvs Cara and Désirée than on cvs Romano and Record (Whitehead, 1993; Whitehead *et al.*, 1995 (in press)). Choice of susceptible cultivar may, therefore, significantly influence soil infestation levels.

Cultivars also differ in their tolerance of nematode attack, independently of their resistance/susceptibility status. This may be expressed in a larger Pt value and/or a shallower regression of yield against nematode number in a tolerant cultivar (Evans & Haydock 1990). Tolerance in a susceptible cultivar will increase nematode multiplication, so it is more usefully linked with full resistance, lessening the decrease in Pi needed for satisfactory crop production. If the resistance base is narrow, it may promote increase of other pathotypes. Tolerance of the two species of potato cyst-nematode may differ in a cultivar (Trudgill & Cotes, 1983). Maris Piper, generally tolerant of attack by *G. rostochiensis*, has shown intolerance to this species in certain conditions. Susceptibility to *Verticillium dahliae* may affect tolerance to potato-cyst nematode attack (Evans, 1987).

Trap cropping

The numbers of root endoparasitic nematodes in the soil, especially cyst nematodes, may be diminished by growing potatoes long enough to allow most juveniles to hatch and invade the roots, the plants then being uprooted and destroyed so that no new eggs are produced. Early harvested first early potatoes have such a trap cropping effect; harvested a little later they may still permit little nematode multiplication (Webley & Jones, 1981). The very tolerant cv. Cara, grown in spring or early summer, can decrease heavy infestations of *G. pallida* (40-465 eggs g^{-1} soil) by 75% or more in about six weeks. This technique could be used on "set aside" fields or could be followed by a late planted crop, such as peas, carrots or a brassica (Whitehead, 1994).

Soil solarization

Numbers of cyst-, root-knot and root-lesion nematodes have been greatly decreased in the topsoil by covering it with one or two layers of clear polyethylene film and allowing the sun to raise soil temperature sufficiently to kill the nematodes. The technique works best in hot climates, where kill in the topsoil may equal that achieved by a soil fumigant. In temperate climes, lethal temperatures may only be attained in the top few centimetres of the soil. In New York State, U.S.A., solarization killed about 97% of *G. rostochiensis* eggs in the top 10 cm in 1981 but far fewer in the cooler summer of 1982 (LaMondia & Brodie, 1984).

Nematicides

Potato nematodes can be killed directly by soil fumigation or paralysed by incorporating nerve-poisonous non-fumigant nematicides in infested soil. The percentage of nematodes that must be killed or immobilised to prevent crop injury is -

$$\frac{100\ (Pi - Pt)}{Pi}$$

If Pt is 5 eggs g soil, 75% kill is needed at Pi = 20 and 95% at Pi = 100. If all the eggs hatch when potatoes are grown, the "kill" needed to prevent nematode population increase (i.e. no. of eggs at harvest = no. of eggs in spring) is -

$$100\ (1 - 1/a)$$

where "a" is the multiplication factor. As a proportion of cyst-nematode eggs does not hatch when potatoes are grown, the equation becomes more complex and rather larger "kills" are needed for non-fumigant than for fumigant nematicides (Table 4), because non-fumigants do not kill eggs within the cysts, so carry-over of viable old eggs is larger than after a soil fumigant.

TABLE 4. Percentage "kill" needed to prevent cyst-nematode population increase when potatoes are grown (derived from model of Jones & Perry, 1978).

Notional Pi	Nematode increase factor (a)	All eggs hatched	"Kill" (%) 70% eggs hatched	
			fumigant	non-fumigant
20	10	90	74	80
10	20	95	86	90
2	50	98	94	96
<1	100	99	97	98

With currently available nematicides - 1,3-dichloropropene mixture (Telone II), some organophosphates (e.g. ethoprophos (Mocap)) and the oximecarbamates (aldicarb (Temik) and oxamyl (Vydate)) - "kill" greater than 90% is rarely attained at commercial dosages. "Kill" is greatly decreased if the nematicide is incorrectly applied to the soil or if the soil is very organic (fumigants and organophosphates) or has a high pH (non-fumigants). Nematicides therefore prevent injury by small or moderate infestations of cyst-nematodes but control cyst-nematode increase best when Pi is fairly large. The non-fumigant nematicides are usually most effective when evenly mixed with the top 15 cm of the soil and less effective or ineffective when applied with the seed. Mixing the granules with the top 30 cm of the soil during stone and clod separation over-dilutes the nematicide. In temperate soils not subject to heavy rainfall in the spring and early summer, non-fumigant nematicides incorporated in the top 15 cm of the soil have little effect on nematodes deeper than 20 cm in the soil, because there is usually little or no downward movement of soil water. In sandy or silty loams, however, nematodes may be killed to a depth of 40cm or more by 1,3-dichloropropene. Few nematodes are killed in the top 5 cm of the soil because the gas usually escapes too rapidly from this layer. Covering the soil with polyethylene film prevented this and contributed to a high percentage kill of *G. pallida* eggs by 1,3-dichloropropene (Whitehead *et al.*, 1979). Combined use of fumigant and non-fumigant nematicides may lessen damage to roots and tubers. Nematicides should not be applied frequently to the same soil because the risk of accelerated (microbial) degradation of the toxicant is increased (Suett & Jukes, 1988).

G. rostochiensis has been well controlled in England by aldicarb or oxamyl at 5.6 kg a.i. ha^{-1} well mixed with the top 15 cm of the soil at planting (e.g. Moss *et al.*, 1976). Nematode increase was reduced, on average, by about 90%. Comparable treatments in the same range of soils for *G. pallida* have been more variable, averaging 50% reduction in nematode increase. Of all the factors examined, which might explain this differential effect, the generally slower rate of hatching of *G. pallida* seems the most likely. If the soil remains moist, *G. pallida* juveniles hatching after degradation of the nematicide may invade and multiply in roots and tubers. Control of *G. pallida* therefore depends on the use of additional measures. Against this species, non-fumigant nematicides should be used at full commercial rates and economies in nematicide usage should be achieved through lengthening of rotations and use of supplementary control measures. Halving the dosage of nematicide may greatly increase nematode multiplication.

Integrated control

Repeated use of a single control measure may select resistant or virulent nematodes from a field population, so successful control is more likely in the longer term from combined use of different measures. The resultant population (Pf) is then the product of the fractions of the initial population left after each independent control measure. For potato cyst-nematodes, for example,

$$Pf = Pi\ (Fo)^a\ (Fr)^b\ (Ft)^c\ (Fs)^d\ (Fn)^e,$$

where Fo, Fr, Ft, Fs and Fn are the fractions left after a non-host crop, a resistant cultivar, a trap crop, soil solarization and a nematicide, respectively, and a-e are the

number of times each measure is used. If Fo = 0.75, Fr = 0.25, Ft = 0.25, Fs = 0.2 and Fn = 0.2 (soil fumigant), a population density (Pi) of 100 eggs g^{-1} of soil would be reduced to < 0.2 eggs g^{-1} soil. With full resistance to *G. rostochiensis*, a simpler programme, using non-host crops, a resistant crop and a nematicide, is effective, e.g. -

$$Pf = 100\ (0.75)^4\ (0.25)\ (0.2) = 1.6$$

With "a" = 50, Pf would then be 80 eggs g^{-1} soil after a susceptible potato crop had been grown and full population control would have been achieved. The same result would be achieved for *G. pallida* by substituting a trap crop for the fully resistant potato, which is not yet available for this species. However, in organic soils in which soil fumigants are much less effective, a nine or ten year break from potatoes would also be needed. Alternatively, keeping the break at four years would require use of a trap crop and a partially resistant or slightly susceptible cultivar grown with a granular nematicide to minimise crop injury and lessen nematode multiplication. When Pi is very much larger than 100, all available control measures, including very long rotations, are needed.

IMPROVING CONTROL OF POTATO CYST-NEMATODES

Although both species of potato cyst-nematodes can be controlled by customised programmes, several advances can be envisaged.

Longer rotations and better control of volunteers should increase decline of *G. pallida* and benefit tuber quality. Regular monitoring of soil population densities and identification of the species present are essential to appropriate integrated control programmes, and monitoring of this type may be easier to achieve with modern immunological techniques (Evans *et al.*, 1995 (in press)). As *G. pallida* is harder to control than *G. rostochiensis*, cultivars resistant to the latter but susceptible to the former should be avoided in mixed infestations in which *G. rostochiensis* predominates, although market forces may dictate otherwise. More commercially acceptable cultivars resistant to *G. pallida* are needed. The exclusion of exotic pathotypes of both species is essential if current breeding effort is not to be wasted in the long term. The use of gas-tight, preferably biodegradable, soil mulches could greatly increase the percentage of potato cyst-nematode eggs killed by 1,3-dichloropropene in non-organic soils. Effective non-fumigant nematicides of greater persistence than aldicarb or oxamyl would be valuable against *G. pallida*. Better methods of incorporating granular nematicides in the seedbed should be adopted. Solar soil heating and trap cropping are environmentally friendly techniques worthy of use in appropriate situations, as would be biological control should it become practical. Whatever control measures are employed in the future, they should be used in concert to prevent rapid selection of virulent or resistant individuals.

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THE USE OF APHID SUCTION-TRAP DATA IN FORECASTING THE INCIDENCE OF POTATO LEAFROLL VIRUS IN SCOTTISH SEED POTATOES

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ABSTRACT

A preliminary model using data on the content of potato leafroll virus in Scottish seed potato crops observed during classification inspections, indicates that the virus level in any year is strongly correlated with the quantity of virus during the preceding year. The correlation can be improved by including data on the abundance of *Myzus persicae* caught during the previous year in the suction traps at Dundee and East Craigs (Edinburgh). The inclusion of data on the total numbers of *M. persicae* caught during the whole year at East Craigs provides a better correlation with the observed data than does the use of *M. persicae* data from Dundee, or the use of data from either trap site for the early summer period (up to 1 August). Multiple regression models support the conclusions of the simpler models but do not provide any significant improvements in the description of the observed data. This work demonstrates the importance of using aphid data from the previous year, indicating that observed symptoms result from virus spread during the previous season. It also questions the importance of *Macrosiphum euphorbiae* in spreading leafroll. The importance of these models to the aphid control measures recently introduced into the Scottish Seed Potato Classification Scheme is discussed.

INTRODUCTION

An important factor for the Scottish seed potato industry has traditionally been that potatoes grown in Scotland are normally less infected by aphid-borne virus diseases than those grown in England (Turl, 1981) and in most other seed potato growing areas within Europe. However, it has been observed that "every few years the Scotch seed potato crop is liable to fall short of the high standards that are aimed at" (Salaman, 1949). To maintain the high reputation for virus health of Scottish seed potatoes, tolerances for virus diseases were introduced in 1932 into what is now the Seed Potato Classification Scheme (SPCS) (Todd, 1961).

Prior to 1992 the incidence of virus disease in crops within the Scottish SPCS was assessed solely by visual inspection. Evidence that supplementary information on the virus content may sometimes be necessary became apparent during the 1990 and 1991 growing seasons when a small proportion of the daughter crops grown for ware in England failed to meet the requirement for basic seed of no more than 4% of the growing plants exhibiting symptoms attributable to virus infection. In addition, the number of seed crops in Scotland

which had been either withdrawn from the scheme or classified as 'No Grade' because of virus was unacceptable in those years.

Against this background changes were made to the SPCS in 1992. The aims were to improve the control of the aphid vectors of the viruses and to provide additional support to classification by backing up the visual inspection with a supplementary post-harvest test when the risk of virus spread is high. The option of introducing routine post-harvest tuber testing for all seed crops was rejected. Such testing would greatly increase the cost of the SPCS, and the generally low virus levels in stocks do not justify routine testing. The major emphasis of the aphid control measures is in two areas: 1) monitoring of crops for potato aphids to determine when an aphid control alert (spray warning) should be issued; and 2) inspecting the crops, following the issue of an aphid control alert, to ensure that aphid control measures have been effective. When aphid numbers on a growing crop exceed a prescribed population, that crop is deemed to be at risk and post-harvest testing becomes a necessary requirement for classification.

A forecast of the date of the first flight of *M. persicae,* as indicated by its first occurrence in the suction trap catches, forms the basis of a recommendation on the need for early season aphid control using insecticidal granules at planting. The forecast is based upon the mean screen temperatures for the months of January and February (Bale *et al.*, 1988). This forecast is also valuable for timing the start of aphid monitoring in the seed potato crops. As the early season flight of *M. persicae* in England is usually around 3-4 weeks before that in Scotland (Harrington *et al.*, 1992), data collected by the Rothamsted Insect Survey from suction traps in England can be used to provide additional information on the timing of the first flight in Scotland.

Increased understanding of the epidemiology of aphid transmitted potato viruses will help to maximise the effectiveness of these aphid control measures. In this paper we concentrate on the potential to make accurate forecasts of the incidence of aphid-borne virus within the Scottish seed potato stocks, using aphid data from the suction traps. If virus levels can be predicted, appropriate measures can be included within the SPCS to ensure that disease levels are kept within acceptable limits. Whilst it is also of great importance to understand the epidemiology of the various strains of potato virus Y, this paper concentrates on potato leafroll virus (PLRV) which has far fewer potential vectors and so represents a more simple system.

METHODS

Potato leafroll virus levels in potato crops

Records of the virus content of classified seed potato crops in Scotland, assessed by visual inspection of the growing crop, are produced annually by the Scottish Office Agriculture and Fisheries Department (SOAFD). Since the 1960s, the total area of the crop in each year has been broken down into a range of categories from nil to 'over 2%' virus content. As each individual category covers a range of virus content, the data were weighted according to the minimum levels of virus for each category and pooled for all varieties and

seed potato grades to produce a single measure of PLRV content for each year. The major sources of error when using such data as an overall measure of the virus content are likely to come from the omission of records on virus from crops withdrawn from the scheme and subsequently grown on for ware; from ware crops; and from diseased plants which have been removed from the growing crop prior to inspection. Changes in the popularity of potato varieties with time would also be expected to have an effect on the virus data through changes in the proportions of resistant and susceptible varieties in cultivation.

Quantifying the PLRV content in this way uses more of the information available within the SPCS statistics than two possible alternatives: using either the data for the percentage of crops (by area) in which any virus was detected for each year (Turl, 1987), or the percentage of crops rejected from the SPCS for PLRV. Neither of these alternatives take into account the incidence of virus within the crops. The data produced by the method advocated in this paper are closely correlated with the data obtained using the method of Turl ($r = 0.936$) and data from the area of crops rejected for PLRV ($r = 0.991$).

Aphid data

The data on the annual abundance of the aphid vectors of PLRV are taken from the catches of the two 12.2m high suction traps operated continually at Dundee and East Craigs since 1967 and 1969 respectively. These suction traps are close to the major areas of Scottish seed potato production and form part of the Rothamsted Insect Survey network (Macaulay *et al.*, 1988). Only data for the aphids *Myzus persicae* and *Macrosiphum euphorbiae*, the major vectors of PLRV, are used in this study.

Statistical methods

Models based on simple linear regression using a single variable, usually a product of the measure of virus content and the number of aphids caught in the suction traps, are used to explore the data (Table 1). The variance accounted for by the regression model (adjusted R^2) is used to assess the goodness of fit of the model to the observed data. An F test is used to assess whether changes to the models significantly improve the fit of the model to the observed data. Where significant improvements in the model are indicated a multiple regression model is used, allowing the measures of virus content and aphid abundance to act separately (Table 2).

RESULTS

The incidence of PLRV and the annual totals of *M. persicae* for the period 1969-1993 are shown in Figure 1. The high populations of *M. persicae* between the years 1972-1976 and the consequent increase in PLRV are apparent. The high aphid population of 1989 was followed by an increase in PLRV in 1991.

The statistical relationships between aphid numbers recorded in suction traps and the PLRV content of the Scottish seed potato crop are shown in Table 1. The simplest model (1) does not use data on aphid abundance. It is simply a reflection of the observation that the

virus content of the seed potatoes in one year is largely determined by that in the preceding year. Years with high virus levels are likely to follow other years of high virus and, similarly, if virus incidence is low, there is little chance that virus will increase to high levels in the following season. This model accounts for 67% of the variation in PLRV content and provides a base line against which any model incorporating aphid abundance can be compared.

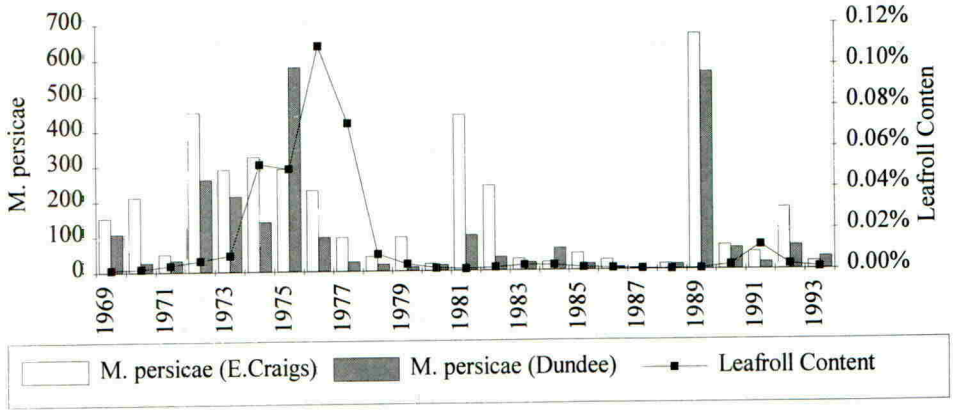

FIGURE 1. Total number of *M. persicae* caught in the East Craigs and Dundee suction traps each year in relation to the leafroll content of Scottish seed potato crops.

TABLE 1. Relationships between the PLRV content in the year (t) ($\ln[PLRV_t]$) and single predictor variables used in simple linear regression models.

Model	Variable Used (A)	Regression Equation	Variance Accounted For	F test c.f. Model 1
1	$\ln\left[PLRV_{t-1}\right]$	y = 0.82A - 1.87	67%	
2	$\ln\left[PLRV_{t-1}.Mp_{t-1}^{EC}\right]$	y = 0.59A - 6.44	82%	$P < 0.001$
3	$\ln\left[PLRV_{t-1}.Mp_{t-1}^{DUN}\right]$	y = 0.61A - 6.82	76%	$P < 0.01$
4	$\ln\left[PLRV_{t-1}.Me_{t-1}^{EC}\right]$	y = 0.69A - 6.33	50%	worse
5	$\ln\left[PLRV_{t-1}.Me_{t-1}^{DUN}\right]$	y = 0.63A - 6.98	51%	worse
6	$\ln\left[PLRV_{t-1}.Mp_{t-1}^{EC}(aug)\right]$	y = 0.55A - 6.12	76%	$P < 0.01$
7	$\ln\left[PLRV_{t-1}.Mp_{t-1}^{DUN}(aug)\right]$	y = 0.63A - 5.65	78%	$P < 0.01$
8	$\ln\left[PLRV_{t-1}.Mp_{t}^{EC}\right]$	y = 0.59A - 6.43	67%	n.s.

In the other models, the predictor variable is produced using the product of the virus inoculum, i.e. the level of PLRV in the previous year ($PLRV_{t-1}$), and the total number of

either *M. persicae* (*Mp*) or *M. euphorbiae* (*Me*) caught in the East Craigs (*EC*) or Dundee (*DUN*) suction traps. In Models 6 & 7, instead of using the aphid totals for the whole year, only the totals prior to 1 August were used (*aug*). In Model 8, the aphid data for the year in which the crop is grown (*t*) has been used and not the aphid data from the previous year, (*t*-1).

Models 2 and 3 indicate that the description of the PLRV content in the growing crop can be improved upon by using a predictor variable based on the product of the inoculum and the numbers of *M. persicae* during the previous year. Incorporation of the *M. persicae* data from East Craigs accounted for 82% of the variation, whilst that from the Dundee trap data accounted for 76%. Both models provided a significantly better fit to the observed data than did Model 1. The fit of Model 2 to the observed data can be seen in Figure 2. Models 4 and 5 indicate that incorporation of data on the abundance of *M. euphorbiae* in the previous year decreased the amount of variation explained. Models 6 and 7 restrict the use of the data on *M. persicae* to aphids caught before 1 August. The regressions from the two sites provided conflicting results. A poorer correlation was produced for the East Craigs data when compared with the correlation using the data for the full year (Model 2). However, when the data prior to 1 August were used for the Dundee trap the correlation was improved, although not significantly. When data on the abundance of *M. persicae* from the same year as the growing crop were used (Model 8), there was no improvement on the correlation obtained with Model 1.

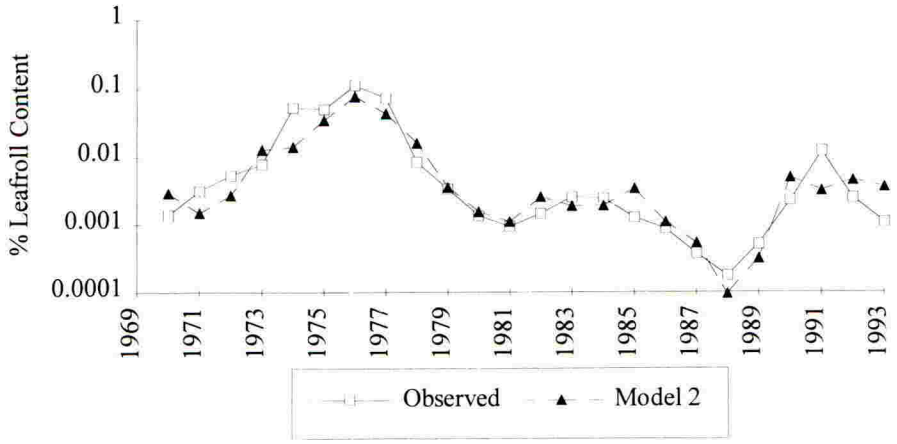

FIGURE 2. The incidence of PLRV in Scottish seed potatoes observed during the growing crop inspections compared with calculated values from regression model 2 (see Table 1).

To investigate whether improved models could be developed using the virus and aphid data independently, rather than combined as a single predictor variable, a series of multiple regressions were conducted. Of the four models detailed in Table 2, none provided a significantly better fit to the observed data than that provided by Model 2 (Table 1). The first model, M1, freed the two variables, $PLRV_{t-1}$ and Mp_{t-1}, rather than constraining them as the product of the two. The fit was not improved. Model M2 supports the observation that the

M. persicae data from the East Craigs trap are of considerably greater value in describing the changes in virus level when compared to similar data from the Dundee trap. Inclusion of the Dundee data into a model including the East Craigs data did not account for any increase in the percentage of the variance explained. The merit of using the *M. persicae* data from both the year of the growing crop and from the previous year is assessed in Model M3. The respective regression coefficients suggest that the current year's data may have some influence on the outcome, with a slight, but not significant, improvement in the fit of the model. Interestingly, the best improvement in any of the multiple regression models is provided by the inclusion of data on *M. euphorbiae* from the East Craigs suction trap (Model M4, $P = 0.13$, n.s.). However, the regression coefficient of -0.50 suggests that this species is not making a positive contribution through its direct involvement in virus transmission.

TABLE 2. Relationships between PLRV content in the year (t) ($\ln[PLRV_t]$) and predictor variables used in multiple regression models.

Model	Regression Equation	Variance Accounted For
M1	$y = 0.65 \ln[PLRV_{t-1}] + 0.52 \ln[Mp^{EC}_{t-1}] - 5.57$	82%
M2	$y = 0.65 \ln[PLRV_{t-1}] + 0.54 \ln[Mp^{EC}_{t-1}] - 0.02 \ln[Mp^{DUN}_{t-1}] - 5.52$	81%
M3	$y = 0.65 \ln[PLRV_{t-1}] + 0.45 \ln[Mp^{EC}_{t-1}] + 0.17 \ln[Mp^{EC}_{t}] - 5.93$	83%
M4	$y = 0.65 \ln[PLRV_{t-1}] + 0.53 \ln[Mp^{EC}_{t-1}] - 0.50 \ln[Me^{EC}_{t-1}] - 3.35$	85%

DISCUSSION

The data on PLRV collected by the SOAFD inspectors during the visual examination of growing seed potato crops can be used to provide a simple descriptive model which can then be used to predict the likely level of PLRV in growing crops during the coming season. This was also observed by Turl (1987). The accuracy of this prediction can be improved considerably by the inclusion of data on aphid abundance, as monitored by the Scottish aphid suction traps. Using simple linear regression models, up to 82% of the variation in virus levels can be accounted for. The use of multiple regression models can increase this figure to 85%, but no statistically significant improvements are achieved.

Data on two potato aphid species known to transmit PLRV were used. Of these, only data on *M. persicae* make a significant improvement to the accuracy of the simple regression model, and the data from the East Craigs trap are of greater significance than those from Dundee. This finding is surprising since the Dundee trap is situated in Angus, the major area for seed potato production in Scotland, albeit in a coastal location. The East Craigs trap is not located close to any major area of seed potato production. The greater emphasis that the

multiple regression model places on the East Craigs data is not an indication that the inclusion of the Dundee data is not statistically significant (the two sets of aphid data are highly correlated), but that little additional information is provided by its inclusion in the model.

The simple linear regression models indicate that suction trap data for *M. persicae* are of far greater significance than similar data for *M. euphorbiae*. When the *M. euphorbiae* data are included in a multiple regression model, a non-significant improvement in the fit of the model is produced. The negative regression coefficient for this species is interesting. As there is a correlation between the *M. euphorbiae* and *M. persicae* data this may not necessarily indicate a negative relationship between the abundance of this presumed vector of PLRV and the resulting virus content. However there is clearly no evidence of a significant positive relationship for a species which is known to be capable of transmitting PLRV (Robert, 1971). The importance of this species as a vector for PLRV has been questioned before, notably by Woodford *et al.* (1983) who observed little spread of PLRV in a field trial in Scotland in which *M. euphorbiae* had colonised early and subsequently reached high population densities.

It may be argued that the totals of *M. persicae* before 1 August in each season should have greater significance in relation to virus transmission since the increasing age of the crop makes the plants both more resistant to the acquisition of PLRV (Cadman & Chambers, 1960) and a poorer source from which the vectors may acquire the virus (Barker & Harrison, 1986). This study provides inconclusive evidence. More of the variance is explained by a model including data for the whole year from the East Craigs suction trap. Using the Dundee aphid catches, the early season data produce the better model. It may be that data sets which include the late summer and autumn flight periods of *M. persicae* at East Craigs can provide additional information on the abundance of this species during the early summer months. For example, if *M. persicae* numbers in potato crops are low throughout the growing season, the late summer or autumn flights are unlikely to be large.

The importance that the models attach to the *M. persicae* data for the previous year (t-1) indicates that the symptoms of PLRV seen at the time of crop inspection are largely a consequence of virus transmission during the previous year, as opposed to acquisition during the current year (primary leafroll). Howell (1973) noted that, in Scotland, "all the plants with symptoms seen by an inspector making his counts within a crop are the results of virus spread the previous year". This work supports this judgement, but the possible importance of the *M. persicae* data for the current year (t) suggests that at least some primary leafroll may be detected at inspection.

A simple model to predict the risk of PLRV transmission in the seed potato crops in Scotland can be used to assess the levels of aphid control measures required for coming seasons. It is also interesting to look back at the two years, 1992 and 1993, in which the current aphid control measures have been in place, and to note that in both years the observed levels of PLRV have been lower than those predicted by the model. This suggests that control measures over these two years have been more effective than the average control measures over the 24 years on which this model has been developed.

ACKNOWLEDGEMENTS

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FUNGUS DISEASES OF POTATO TUBERS AND STRATEGIES FOR THEIR CONTROL

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ABSTRACT

Potato tuber diseases damage seed and decrease the productivity of crops and the quality of stored tubers. Many are tuber-borne and can be avoided by propagating from healthy nucleus stocks of seed. But, as fungal inoculum is common in the farm environment, fungicide treatment of seed is necessary to prevent increase in disease acquired during seed multiplication. Methods of applying fungicides to tubers need to be improved to increase efficiency of use and efficacy of disease control. Resistance to fungicides poses a threat to the continued improvement in crop health and strategies are required for fungicide use throughout seed and ware production. Agronomic practices also contribute to keeping diseases scarce and when integrated with chemical methods currently offer the best chance of decreasing the risk of disease.

WHY CONTROL TUBER DISEASES?

The potato is prone to a variety of fungal diseases that cause damage throughout the 18-month cycle of production from receipt of seed tubers to disposal of stored ware. On seed, rots and blind tubers cause gappy and uneven crops or, if rogued out before planting, loss of planted area. Delay in shoot emergence, altered stem numbers, decreased yield and changes in the number and size of tubers are other penalties of planting diseased seed tubers. Furthermore, diseases transferred to daughter tubers continue to develop during storage; quality declines not only with diseases that rot tubers but also with those that cause superficial blemishes. Whereas rotted tubers are not acceptable in any market, in recent years the common blemishing diseases silver scurf and black dot have halved the value of crops destined for sale in prepacks.

Specifications for seed and ware are unlikely to be relaxed and premiums will continue for the most acceptable crops. Also, among groups of seed and ware producers there is the determination to improve the precision of potato production and to decrease that proportion of the crop which is unsaleable, whether from tuber size or disease. There are therefore ample reasons for improving the control of these diseases.

SOURCES OF DISEASE

We are fortunate that the important tuber diseases skin spot, gangrene, silver scurf, dry rot and black scurf originate from inoculum on seed tubers. Fortunate, because the inoculum is discrete and readily lends itself to control. This is in contrast to inoculum distributed throughout the cultivated soil layer. For this reason, in the early 1960s we suggested that commencing seed multiplication with small quantities of tubers free from fungal pathogens

was the best way of starting a disease control strategy. Results from the early stem cutting progenies were encouraging (Hide, 1978) both from an improvement in health status and from a decrease in outgrades. But, during the later multiplication of commercial seed, pathogens rapidly appeared and there was some justification in questioning whether the Virus Tested Stem Cutting (VTSC) scheme would benefit ware producers. However, recent evidence suggests that disease incidence has decreased during the past 20 years (Carnegie, 1992).

At the time when the VTSC scheme started, seed tubers were considered to provide the major source of inoculum for these diseases, and other sources were thought to be of little consequence. But the experience with VTSC stocks, supported by recent investigations, shows that important amounts of inoculum are present in field soil, on dirty equipment, in dust and soil in stores or are spread in the field, and this is available for infecting healthy crops. Therefore producing clean nucleus stocks is only the start of a healthy multiplication scheme. By halving the number of multiplication years, the current use of mini tubers also helps to decrease the risk of contamination and the increase in inoculum that occurs as seed stocks are multiplied. Much is still to be learnt about the sources and persistence of this inoculum, how it is spread and how it may be avoided, contained or controlled. Also quick and reliable methods are required for its detection.

It seems certain that the apparent early failure to control the tuber diseases with pathogen-free planting material lies in our attempt to impose a healthy regime into an existing farming system. The evidence now available suggests that healthy seed production must be coupled with measures that improve hygiene on the farm and prevent build up of infection acquired during commercial multiplication.

The problem with diseases that originate from soil-borne inoculum is more difficult to solve. Although powdery scab and black dot are seed tuber-borne, it is the reservoir of inoculum in soil that often causes serious disease in many commercial crops. Similarly, stem canker and black scurf can arise from soil-borne inoculum especially in short rotations, and the skin spot fungus persists in soil for several years. Unless sterilants can be developed for eradicating soil-borne inoculum, or fungicides incorporated into the cultivated soil layer, control is likely to depend on cultural factors such as the choice of less susceptible cultivars, the choice of fields with good drainage and earlier harvest date, perhaps combined with healthy seed and fungicide seed treatment (Read, 1991; Burgess & Wale, 1993).

FUNGICIDE TREATMENT OF TUBERS

Applying fungicides to tubers prevents the development of rots and blemishes. The first materials used were inorganic or organic compounds of mercury to prevent damage to treated seed tubers. More recently, fumigation with 2-aminobutane has also been developed to prevent deterioration of seed during storage.

In the work at Rothamsted we set ourselves the task of using fungicides on seed tubers to improve the health of the subsequent crop. Pilot trials showed that thiabendazole applied to tubers immediately after harvest prevented development of gangrene, dry rot, skin spot and silver scurf in store. But also, and more importantly, when previously applied to diseased

seed tubers before planting in 1968, it decreased infections on daughter tubers at harvest (Hide *et al.*, 1969). This confirmed the potential of using fungicides on seed tubers, a technique that is now well established as a method of improving the quality of crops removed from store up to 18 months after the application of fungicides (Table 1). Release of other materials for this purpose followed, including those with specific activity against *Rhizoctonia*.

TABLE 1. Seed tuber treatment with imazalil (I) or imazalil plus thiabendazole (I+T) and silver scurf after storage

		Silver scurf (disease score)		
	Seed Treatment	On seed tubers	On crop tuber	
			Untreated	Treated
1987	I	20	32	24
1988	I	22	51	27
1989	I	2	53	3
1990	-	68	52	-
1991	I+T	42	40	9
1992	I+T	25	50	19

As far as we can determine, disease control depends on depositing the materials onto the skin of seed tubers so that, for instance, *Helminthosporium* (silver scurf) and *Polyscytalum* (skin spot) are prevented from sporulating after planting, and *Rhizoctonia* sclerotia do not germinate. The corollary is that the degree of control is dependent on the proportion of the tuber surface covered with fungicide. Also the materials need to persist throughout the growing season to prevent the escape of inoculum. However, as the current materials do not penetrate deep into tuber flesh, effective deposition has to be followed by careful handling to prevent exposing the underlying untreated infected tissues. Gangrene in stored ware has not been controlled with fungicides applied to the seed tubers.

METHODS OF APPLICATION

In the late 1960s there was little experience of applying fungicides to large propagules such as seed potatoes other than dipping, and methods of application evolved from the experimental use of powders impacted onto tubers, to sprays applied at the end of elevators and then to application over roller tables. However, early spray application seldom achieved good cover on the tubers; a large proportion of the spray was lost to the environment and when used in practice problems were experienced with excessive wetting of the tubers with fall-out from the nozzles, and this led to bacterial soft rot. In an attempt to increase efficiency of deposition we developed an electrostatic applicator (Cayley *et al.*, 1987) and the Microstat is now an accepted option for fungicide application. However, as with all the machines currently available, efficient application does not equate to effective disease control which will also be influenced by the amounts of inoculum, retention of the materials on the tuber periderm and the amount and type of adhering soil.

With the adoption of tuber treatments into normal husbandry it became necessary to investigate why disease control could be so variable (Table 1).

The precise amount of chemical required to control diseases is probably not known because tubers can carry widely differing amounts of inoculum and therefore recommended doses may be insufficient when inoculum is prevalent. Also, the doses currently recommended are given for the weight of tubers whereas it is the surface of tubers that is to be treated; crops of small tubers could be underdosed.

Water is required as a carrier to ensure adhesion and sorption of materials onto the tuber periderm or into wounds, but tubers can carry differing amounts and types of adhering soil that impede deposition onto the target; adherence is necessary as tubers are subsequently handled and deposits lost. The pH of the adhering soil also influences fungicide efficacy. Furthermore, inspection tables were not designed for spray application and modifications are needed for treating tubers to give adequate rotation and avoid depositing the spray onto exposed roller surfaces. It was to define and address these and other problems, to consider operator safety and the standardisation of terms and methods, and to encourage correct use and calibration of equipment that the BCPC Potato Treater Group was set up in 1991 (Ingram, 1994).

FUNGICIDE RESISTANCE

Routine use of fungicides on seed tubers might have been expected to lead to fungicide resistance and in 1985 we first found resistance to thiabendazole in *Helminthosporium* and *Polyscytalum*; results from more recent experiments suggest that it had been present for at least 10 years before we detected it (Hide & Hall, 1993). In common with colleagues in Europe we also found resistance to thiabendazole in *Fusarium sulphureum* (dry rot).

The evidence available suggests that resistance in *Helminthosporium* and *Polyscytalum* develops soon after thiabendazole is applied to seed tubers and its prevalence on daughter tubers increases with further annual applications during seed multiplication, with a consequent loss of disease control. With *Helminthosporium*, resistance was detected in fungal isolates obtained from silver scurf lesions on the surface of seed tubers within a few weeks of treatment. Resistant isolates were as aggressive as sensitive ones and they also persisted for several years in crops where the fungicide was not subsequently used. This suggests that fungicide resistance in a crop could arise not only as a result of treating seed of that crop, but also from fungicide used during the earlier multiplication of the seed, or perhaps from inoculum left in the soil from a previous crop grown from treated seed. Furthermore, the incidence of resistance increased as the fungicide dose increased and, if improvements are made in application techniques, it is likely that the risk of resistance will also increase unless amounts of chemical applied are decreased.

The detection of resistance in these potato pathogens by cultural methods is lengthy. More rapid techniques are required if information on its incidence is to be used by growers in deciding methods of crop management.

The discovery of resistance to thiabendazole stimulated studies on strategies of fungicide usage during the years of seed multiplication so that efficacy is maintained (Burgess *et al.*, 1993). For example, it might be wise to retain thiabendazole for use during ware production and use other materials when required during seed multiplication. Such strategies will need to be

constantly monitored to incorporate new materials into the scheme as they become available and so widen the options for seed treatment. Also, resistance needs to be detected as soon as possible.

In 1975 we used imazalil on seed tubers for the first time and this material, either applied by itself or with others, is an effective alternative for use on seed for controlling most diseases. Furthermore, resistance to thiabendazole is greatly decreased in the presence of imazalil (Hide & Hall, 1993), and to date there have been no reports of resistance to imazalil in the potato tuber pathogens.

WHEN IS THERE A NEED TO USE SEED TREATMENT?

Decisions on whether or not to treat seed tubers currently depend on a variety of factors including the amount and type of disease found on seed tubers, the perceived risk of disease based on previous experience on the farm or field and the intended purpose of the crop. Alternatively, fungicides are sometimes used routinely as an insurance against disease.

If relationships could be established between amounts of disease on seed tubers or inoculum in soil and the severity of disease on daughter tubers, the use of fungicides could be based on risk assessments. In attempting to relate the incidence of infection or disease at different times during the crop cycle we found that, although amounts on crops in August were sometimes related to disease on seed, significant relationships became fewer at harvest and after storage, presumably as crops were influenced by other factors (Adams & Hide, 1980).

TABLE 2. Effect of planting different seed stocks on the same field or on different farms on skin spot after storage, 1975.

Seed stock	Skin spot (% tubers)		
	On seed tubers	On crop tubers	
		Same field	Different farms
1	5	3	28
2	8	5	47
3	16	13	14
4	46	29	51
5	52	24	7

There is no doubt that amounts of disease on the seed tubers do affect the relative severity of crop disease and this can be amply demonstrated by planting samples of different stocks in adjacent plots which are all under the same field influence. Growing samples of the same stocks on different fields demonstrates the modifying effects of the sites. But predicting disease with seed stocks having differing amounts of disease becomes unreliable when they are planted on different sites (Table 2). At each site weather or soil conditions must variously encourage or suppress transmission of inoculum from the seed to daughter tubers and so affect the subsequent establishment of infection and disease development.

Storage conditions also modify disease development, but the reasons for

this may not be straightforward, because widely differing amounts of disease developed in different years when samples of tubers with similar amounts of disease or infection were stored under apparently identical conditions (Table 3). Therefore it is probable that growing conditions, besides affecting spread of inoculum, also influence tuber susceptibility and disease development during the subsequent 6-month storage period.

TABLE 3. Tuber infection at harvest and disease after storage for 6 months, mean of 15-21 crops each year.

	1971	1972	1973	1974
Gangrene				
Harvest	3	6	11	1
Storage	10	1	2	4
Skin spot				
Harvest	58	21	13	13
Storage	18	2	10	25
Silver scurf				
Harvest	25	24	15	23
Storage	5	10	7	42

Before we can base the use of fungicides on assessments of disease risk, it will be necessary to obtain a full understanding of the epidemiology of the individual diseases and the influence and importance of different growing and storage environments. Also, to date all results and observations from experiments have been used to examine relative differences between inoculum, infection and disease in different crops. This could be used in deciding which, from a range of crops, would be those to keep for long-term storage and which constitute a high risk and so should be disposed of quickly. Although much evidence is already available on seed and growth parameters, we are some way from predicting the actual amounts and severity of disease that might develop by harvest or after storage.

SUPPORTING METHODS

Even under strictly controlled conditions, seed treatment with fungicides gives variable results. On farms, this might be attributed to poor application and deposition, to subsequent contamination of treated seed tubers by inoculum in stores or to contamination of daughter tubers in field soil or in stores after harvest. For this reason we have attempted to develop an integrated approach to disease control with chemical and non-chemical methods that continuously influence disease throughout the 18-month crop cycle (Hide *et al.*, 1994).

It is well known that tubers are infected with the fungal pathogens soon after they have been initiated and that amounts of infection increase whilst they remain in soil. By harvesting crops earlier than normal the severity of skin spot, silver scurf, gangrene, black scurf and black dot in store is decreased; but as the increase in infection during growth differs between seasons it is not yet possible to provide optimum calendar dates for haulm destruction and harvest.

Holding freshly harvested tubers in warm conditions (curing) for up to

2 weeks encourages wound healing and is effective in controlling gangrene. However, we found that if the environment is dry (c. 80% r.h.) during the curing period, much less skin spot, silver scurf, black dot and black scurf develops than with normal curing (c.95% r.h., Table 4). This dry curing seems to eradicate fungal infections and prevent formation of *Rhizoctonia* sclerotia. Furthermore it is more effective the earlier crops are harvested, possibly because infections are fewer and they are not well established in tuber tissues. Dry curing is now an integral part of store management on many farms. Similarly, windrowing (2-stage harvesting) may also contribute to disease control by eradicating infections while the tubers are allowed to dry on the soil surface for several hours immediately after harvest. Although earlier harvesting may result in a yield penalty, smaller yields of healthy potatoes are likely to be more acceptable than larger yields of diseased ones.

TABLE 4. Effect of imazalil seed treatment, harvest date and curing conditions on silver scurf after storage at 7°C and 95% r.h. 1988

	Harvest date					
	30 August		29 September		17 October	
Curing r.h.	Nil	Imazalil	Nil	Imazalil	Nil	Imazalil
95	50	25	76	57	88	67
80	23	9	55	24	73	53

There are also prospects of integrating the use of fungal and bacterial antagonists with cultural methods for controlling tuber diseases. For example in the Netherlands black scurf was controlled in a system of green crop harvesting (Van den Boogert *et al.*, 1994). After mechanically pulverising the haulm, tubers were harvested and then covered with soil; separating the haulm from the tubers prevented *Rhizoctonia* sclerotia from developing, and control was improved when conidia of *Verticillium biguttatum*, a fungus antagonistic to *Rhizoctonia*, were sprayed onto tubers before covering them with soil. Promising results were also obtained with fungi and bacteria antagonistic to *Erwinia*.

CONCLUSIONS

While inoculum remains common in the farm environment, measures will be required to maintain health of seed tubers and prevent damage in subsequent crops. It is expected that amounts of disease on commercial seed will continue to decline and this might be accompanied by a decrease in the demand for fungicides. With strict regimes and attention to detail some enthusiastic growers are now producing seed stocks with minimal amounts of disease without the use of fungicides.

However, it is unrealistic to expect that the tuber-borne diseases can be completely eliminated throughout the years of seed multiplication and ware production. The problems of maintaining health become greater as the scale of production increases. Therefore we shall have to anticipate small amounts of disease, with our efforts aimed at keeping these at a low level.

For the foreseeable future seed treatment fungicides will be required to decrease the risk of disease in high value crops. An integrated approach using

chemical and non-chemical methods promises to offer the most reliable system for maintaining the value of crops in a variety of situations and years. But with further information on inoculum and its eradication and on disease epidemiology it will be possible to pinpoint weaknesses in our production systems and to address these weaknesses by modifying strategies.

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MANAGING LATE BLIGHT OF POTATOES IN THE 1990s

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ABSTRACT

Late blight of potatoes caused by the fungus *Phytophthora infestans* remains potentially the most damaging disease of potatoes. The disease may develop rapidly under favourable environmental conditions and with only protectant fungicides available and limited cultivar resistance, this will remain the situation for some years to come.

Recent research at SAC Auchincruive has highlighted the importance of stem infection in the epidemic development of *P. infestans* and in particular in the initiation of tuber infection. High risk crop growth stages have been identified. A programmed approach to fungicide selection is recommended with active ingredients chosen to meet these high risk growth stages.

Non-chemical components of late blight management are considered and a unique diagnostic test kit for the on-site detection of *P. infestans* is described.

INTRODUCTION

Late blight of potatoes caused by the fungus *Phytophthora infestans* first appeared in Europe in 1845. Some 150 years on, *P. infestans* remains potentially the most damaging disease of potatoes, posing an annual threat for growers. Although advances have been made in developing potato cultivars with higher levels of resistance to *P. infestans*, there are still none which have sufficient resistance to prevent the disease developing given conditions favourable for its development. Similarly, whilst the fungicides for late blight control have become more varied and sophisticated, they are essentially still only protectant in their activity having limited ability to prevent disease development once it has appeared in the crop.

It is against this background that growers must approach the control of *P. infestans*. The aim of late blight control must be to delay the onset of disease long enough to obtain maximum yield and to take appropriate action to minimise the risk of tuber infection. This cannot be achieved by the use of fungicides alone. Rather, it must result from a comprehensive approach involving a clear understanding of the disease, careful attention to

management and correct fungicide usage. It is this failure to look for an integrated system which often leads to disappointment with the control achieved.

Tuber infection is of particular concern to growers. Even a small percentage of infected tubers can result in rejection of crops grown for speciality markets. In stores, blighted tubers may be the entry points for secondary soft rotting bacteria leading to losses out of all proportion to the original incidence of *P. infestans*. In recent seasons, there have been reports of high levels of tuber infection (30-40%) in the apparent absence of foliar blight (Fairclough *et al.*, 1993).

STEM AND TUBER INFECTION

Research conducted at SAC Auchincruive between 1990 and 1993 has highlighted the importance of stem infection in the occurrence of tuber blight. Some of the results are summarised below.

The growth of *P. infestans* in the stems of potato plants

Materials and methods

In glasshouse experiments, the stems of cv. Home Guard (5 wk old) grown in pots of compost were inoculated with *P. infestans* by placing a 5 mm x 5 mm piece of filter paper bearing approximately 500 sporangia around the base of the main stem approximately 5 cm above compost level. The filter paper was retained in position with Parafilm. Thus prepared, the plants were maintained at 10°C with 16h light in each 24h cycle. The progression of *P. infestans* up the stem was determined 7 days after inoculation by sectioning the stems transversely at 10 mm intervals from the inoculation point and conducting an ELISA test (Clark & Adams, 1977) on a ground preparation of each section. No symptoms were visible at the time of assessment.

Results

After only 7 days, *P. infestans* could be detected by ELISA some 60-70 mm above the inoculation point (Fig. 1). The highest concentration of the fungus was at 30-40 mm.

Histological studies showed that the mycelium was restricted to the epidermal tissues of the stem.

The development of stem infection

Materials and methods

Potato seed cv. King Edward was planted in field plots at SAC Auchincruive on 15 May 1992. On 10 July and at 10 day intervals thereafter, the plots were sprayed with mancozeb (1360g ai/ha). On 17 July, selected main stems were inoculated using the procedure detailed in the previous experiment. The inoculum load was 200 sporangia per 5 mm x 5 mm piece of filter paper. The trial was replicated and treatments arranged in a randomised block design. The development of stem lesions was determined 10, 25 and 42

days after inoculation. At the second assessment, the percentage of inoculated stems which had sporangia of *P. infestans* on them was also determined.

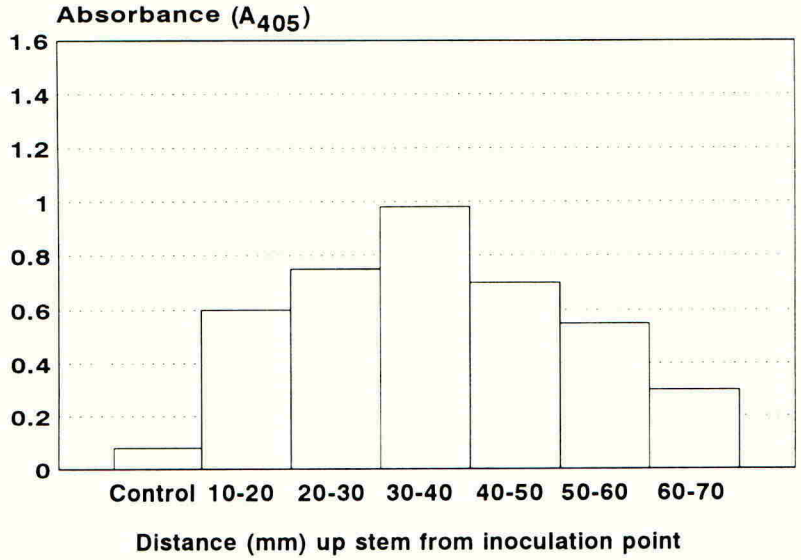

Fig. 1. The growth of *P. infestans* (in mm) up potato stems 7 days after point inoculation 5cm above the soil surface.

Results

Stem lesions were recorded on plants 10 days after inoculation (Table 1). These continued to develop inspite of a 10 day fungicide programme. By mid-August, some 73% of stems were actively producing sporangia.

Although no foliar blight was recorded in the trial, 15% of tubers from inoculated plants were infected. Tubers from uninoculated plants had no infection.

Activity of fungicides against foliar, stem and tuber infection by P. *infestans*

Materials and methods

A replicated field trial of potato cv. Kingston was established in 1991 and sprayed at 10-14 day intervals from 3 July. The chemicals used were mancozeb (1360g ai/ha), fentin hydroxide (266g ai/ha), metalaxyl + mancozeb (150g + 1350g ai/ha) and oxadixyl + cymoxanil + mancozeb (200g + 80g + 1400g ai/ha). An unsprayed control was included. The incidences of foliar and stem blight were assessed on several occasions during the season. The onset and development of tuber infection was monitored by test diggings (324 tubers per treatment) at 10 day intervals from early July until the end of September.

Results

Foliar and stem infection: Fungicides differed in the protection they provided against stem infection (Table 2). The incidence of foliar infection was not directly related to the severity of stem blight. Fentin hydroxide provided better control of foliar and stem blight than mancozeb. Its performance in this trial as a season-long programme was similar to that of the three-way mix.

TABLE 1. The development of stem lesions on field-grown potato cv. King Edward, point inoculated with *P. infestans* on 17 July 1992.

Days after inoculation	Mean lesion length (mm)	% inoculated stems sporulating on 12 Aug
10	32	-
26	107	73
42	111	-

TABLE 2. Activity of fungicides against foliar and stem infection by *P. infestans*

Treatment	% stem area diseased on 29 Sept.	% foliage blight on 29 Sept
Untreated	98	28
mancozeb	48	22
fentin hydroxide	3*	13
metalaxyl + mancozeb	28	24
oxadixyl + cymoxanil + mancozeb	13*	9*

* significantly less blight than the mancozeb treatment

The ability of fentin hydroxide to minimise stem infection was confirmed in a second trial in which fentin hydroxide was associated with significantly lower levels of stem infection than mancozeb.

Tuber infection: The onset of tuber infection and the subsequent increase in incidence with time varied markedly between treatments (Fig. 2). Mancozeb effected a delay in tuber infection of around 20 days compared with the unsprayed but ultimately the incidence was higher than in the unsprayed. Fentin hydroxide and the three-way mix were

most effective in reducing the incidence of tuber infection and the two-way mix was intermediate between these two and the mancozeb treatment.

In other fungicide trials (Bain, unpublished), conducted between 1987 and 1991, fentin hydroxide applied throughout the season or as the last two applications consistently gave the lowest incidence of tuber blight.

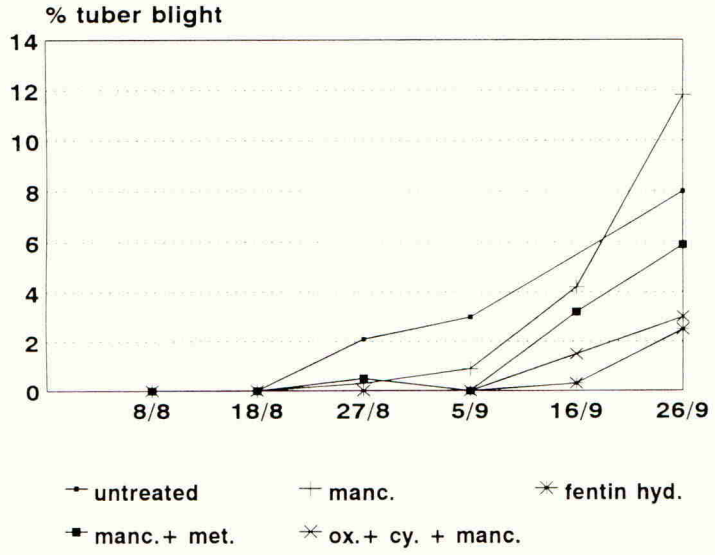

Fig. 2. Fungicide treatment and the onset of infection of potato tubers by *P. infestans*.

TARGETING HIGH RISK GROWTH STAGES

It is possible to identify periods during the growth of the potato crop when it may be particularly vulnerable to attack by *P. infestans* should various biotic and abiotic factors coincide. The complexity of the environment around the crop and its influences on the epidemic development of *P. infestans* have been dealt with in a detailed review by Harrison (1992). The recognition of such 'danger periods' can assist in the recommendation of fungicide protection programmes which utilise the features of the various fungicide groups.

Between crop emergence and the foliage canopy closing over

At the early growth stages of the crop, adequate protection of plants against infection is particularly difficult because of the rate of growth of the crop. Infection of the lower plant parts is extremely difficult to control once the canopy has closed over. Sporangia produced in the favourable environment below the canopy may be washed down into the soil over an extended period creating many opportunities for tuber infection.

It is during this early period of rapid growth that the properties of the two-way mix (e.g. metalaxyl + mancozeb) or three-way mix (e.g. oxadixyl + cymoxanil + mancozeb) systemic fungicides are most valuable. However, there may be a question mark over the two-way mix products, the systemic components of which belong to the phenylamide group (e.g. metalaxyl, oxadixyl and benalaxyl). Their efficacy may be reduced in the presence of phenylamide-resistant *P. infestans*. It is sensible to assume that phenylamide-resistant blight will be present in the population and that its proportion is likely to increase with repeated application of two-way mix phenylamide fungicides. This scenario should be borne in mind when selecting products and in deciding on the number and timing of the applications of the selected product.

Mid-season

Whilst the canopy is complete, the aim should be to maintain adequate fungicide coverage. Adhering to the recommended intervals for the prevailing risk probably is more important than the product selected. A contact fungicide or a cymoxanil + mancozeb mixture should be adequate. The 7 day minimum spray interval for fluazinam makes it an attractive option particularly under changeable weather conditions.

Between the last application of fungicide and the application of a desiccant

When the foliage canopy begins to open up as the haulm collapses, disease at the base of the plant can develop rapidly. In particular, stem lesions may begin to sporulate profusely as the tissue senesces (Fairclough, unpublished). This particular 'danger period' often is associated with an extension in spray application intervals (Bain & Holmes, 1990). Maintaining adequate cover is essential at this time and a tin-based product should be applied no more than 7 days after the last application of a non-tin product.

During desiccation

With even the most effective desiccant, there may be a considerable delay before complete death of the haulm. Sporangia produced on stems are readily washed down into the soil and may contaminate or infect tubers. Continued protection with a tin-based material throughout the burning down period is essential to prevent tuber blight.

Between desiccation and lifting

Sporangia of *P. infestans* may survive in soil for up to 14 days. Early lifting may result in contaminated soil adhering to tubers being carried into store. Depending on lifting and storage conditions, this may lead to tuber infection. Observing the traditional 14 day interval between the complete death of the haulm and lifting minimises this risk.

NON-CHEMICAL CONTROL MEASURES

Management

Careful attention to aspects of management, reinforced by an appreciation of the life cycle of the causal agent complements any fungicide programme. Preventing the growth of haulm on potato dumps, destroying groundkeepers and using disease-free seed may delay the onset of an epidemic. Thorough burning-off and a 14 day interval before lifting will reduce the incidence of tuber infection. Where possible, potato cultivars with a higher degree of foliar or tuber resistance to *P. infestans* should be chosen.

Disease forecasting

Attempts to develop a method of forecasting the initial occurrence of late blight and subsequent 'high risk' events began some 50 years ago with the Beaumont Period (Beaumont, 1947). More sophisticated systems such as BLITECAST (Krause *et al*, 1975) and negative prognosis (Schrödter & Ullrich, 1966) have since been developed. The basic forecasting systems are based solely on temperature and humidity parameters, more sophisticated ones incorporate spore trapping data, weathering of fungicides and varietal resistance. Attempts to predict disease effectively have been frustrated by the inherent threat which the disease poses each season and the variability in environmental conditions within relatively small geographical areas. In-crop monitoring is expensive but undoubtedly is a more satisfactory approach. Forecasting may not be widely accepted until curative fungicides are available.

Diagnostic tools

Rapid on-site diagnosis of lesions which are suspected of being due to *P. infestans* can provide a valuable management tool for farmers. Such a test was introduced into the UK in 1993 and is marketed under the name of ALERT by ADGEN Diagnostic Systems. The diagnostic test kit is based on the enzyme-linked immunosorbent assay (ELISA). In the test, antibodies trap the target pathogens on a membrane in a pill-box sized detector unit. The sequential addition of more of the same antibodies to which an enzyme has been attached (conjugate), a rinse solution and finally the substrate on which the enzyme acts produces a colour change if the pathogen is present. The detector unit also includes positive and negative checks to ensure that the test has been conducted correctly. The whole procedure takes 10 minutes and can be done in the field.

Extensive evaluations of the *Phytophthora* test kit on potatoes were conducted in 1993 (Holmes, unpublished). Samples of leaf, stem and tuber tissue were collected from potato crops throughout the UK. Tests with the kit were conducted in the field and compared with conventional laboratory identification procedures.

The kits proved to be effective in detecting *P. infestans* in all types of potato tissue. Non-sporulating lesions provided strong positive colour reactions in the detector unit. There was complete agreement between the field tests and the laboratory procedures.

CONCLUSIONS

Infection of potato stems by *P. infestans* is a more important aspect of the epidemiology of the pathogen than recognised previously. It is a key factor in the incidence of tuber infection and could account for reports in recent years of high levels of tuber infection in the apparent absence of foliar blight. Also, the relative performance of fungicides against stem blight and tuber blight differs from that against foliar blight.

This information, combined with the recognition of high risk crop growth stages can promote the most effective selection of available fungicides. Integrated management of late blight will be the most efficient option for the foreseeable future.

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Session 4B

Modern Approaches to Toxicity Testing

Chairman	Dr I F H Purchase
Session Organiser	Dr R J Harling
Papers	4B-1 to 4B-4

THE USE OF EXPERIMENTAL ANIMALS: WHAT ARE THE ETHICAL ISSUES?

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ABSTRACT

The use of animals for the safety evaluation of pesticides is a legal requirement which is based in ethical concerns for the safety of people exposed to them. There is a further ethical concern created by the use of animals for experimental purposes. The philosophical approach to this moral dilemma is discussed. Arising from the competing ethical concerns, an increasingly detailed scientific justification of the choices made in the design and conduct of animal experiments is required, so that the interests of both humans and animals are served.

INTRODUCTION

Within the context of the International Agrochemicals Industry, experimental animals are used primarily for the assessment of the hazard to human health of agrochemicals. This activity is relatively recent, with legislation developed over the last 50 years. Ethical concern for the welfare of animals has been many centuries in the making but has become more of a public issue over the last century. Indeed the first legislation directed particularly to the protection of animals was the UK Cruelty to Animals Act of 1876. This paper examines the ethical issues and legislation associated with Regulatory Toxicology Testing by the Agrochemical Industry on an international basis.

MORAL ISSUES

The Utilitarian view of the use of animals in experiments is that the costs involved in their use is outweighed by the benefits which accrue to mankind and, in some cases, to animals. Animal experiments have led to an increasing body of knowledge which in turn has resulted in inestimable benefits to society, particularly in the medical sciences but also in the invention and development of products in health care, industry, agriculture and the home.

The Utilitarian view presents the experimenter with a moral dilemma often referred to as the **tragic conflict**. On the one hand it recognises that to experiment on animals may inflict pain or suffering on those animals. On the other, as such experiments are frequently in the short or long term of great benefit to mankind, can we deliberately avoid taking the steps which may lead to the prevention of much pain and suffering in humans? We face the tragic conflict of choosing between two actions, either of which may result in pain and suffering to living beings. This tragic conflict may be exemplified within the context of the use of

animals for the testing of pesticides. If we develop new pesticides, it will inevitably result in the use of hundreds of animals to ensure their safety in use, with the attendant consequences in terms of pain and distress that the animals may suffer. On the other hand, if we do not develop the pesticide food production will be less and of a lower quality than it might have been and consequently people may suffer from malnutrition or starvation.

Animal rights philosophers advance an alternative view. Their argument is that animals can feel pain and distress as can man. Indeed it is because animals are so like man that they are useful for experimental purposes. If they are so like humans, why should they be treated so differently? If we use them for experimental purposes, we disregard their interests and hence violate their rights.

The argument that animals have rights raises the question of what constitutes the main morally relevant difference between animals and man which allows the continuation of animal experiments? It cannot be argued that it is due to the inability to suffer, because their is ample evidence that animals can and do suffer pain and distress. The main philosophical defence advanced is that animals do not have a self-consciousness and rationality possessed by humans. Being self-conscious involves the ability to relate one's circumstances and experiences to an understanding of one's self as an enduring individual. That is with the ability to make judgements about the past, the present and the future which in turn is dependent on the ability to distinguish between evidence which has a bearing on the past, the present and the future. It requires the capacity of general knowledge and the ability to conceptualise facts not immediately present, the presence of a complex symbolic system to articulate and distinguish these facts and a high level of intelligence.

The animal rights movement often advances the argument that, if it is acceptable to carry out experiments on animals because they do not have self-consciousness, it must be axiomatic that, for example, disabled people and young children who do not (yet) have self-consciousness can equally be the subjects of experiments. This argument questions the concept of rights. Who decides that an animal or individual has rights? If we agree that the morally distinguishing feature which differentiates human from animals is the possession of self-consciousness, it brings with it the concept that rights are conferred upon other individuals by common consent of those in the community; in the case of human rights, it is possible for humans to decide on the morally relevant rights to confer on each other. By so doing not only do they benefit from those rights but they also carry the obligation to confer those rights to others in the society. Animals cannot enter into the debate on this issue and thus carry no obligations; in consequence it would be argued that they do not have any automatic benefits. An extension of this argument allows the society to offer individuals, who by reason of age or infirmity cannot enter into the contract for rights, full access to rights without having to contribute to the obligations which they normally carry. A further consequence would be the imperative for us to extend to

animals the right to suitable welfare and humane treatment as part of the overall balance of rights in society.

ETHICS IN TOXICOLOGY

Toxicology in the Agrochemicals Industry is a pragmatic science with the principle aim of protecting the health of those who might become exposed to the chemicals concerned. On the one hand this requires that the toxicologists satisfy themselves that the product is safe. Subsequently the regulatory authorities must be satisfied that it is safe in use. Thus there is a real benefit to human health in the work that they do.

On the other hand, animal experiments are at the heart of pesticide safety assessment, and hence toxicologists carry responsibility for the welfare of the experimental animals used. Thus there are ethical concerns both in protecting human health and in animal welfare - the tragic conflict described above. In the case of agrochemicals, the benefits of production of higher quality food in greater quantity on the health and welfare of mankind is substantial providing a moral imperative in support of the use of experimental animals for testing of pesticides.

ETHICS, SCIENCE AND THE LAW

Toxicology testing is different from many other uses of experimental animals in that it is mandated by law. These chemicals, in common with many other categories of chemicals, must be tested to certain standards before they can be sold for use as pesticides; in addition re-testing is often required to assure safety of products which have already been on the market for some time.

At the same time, the use of experimental animals is controlled by law. In the case of the European Union (through Directive 86/609/EEC) and the UK (through the Animals [Scientific Procedures] Act 1986), the legislation has taken into account many of the ethical issues raised in connection with toxicity testing. For example, the concept of seeking alternatives by Refinements, Replacements or Reduction of the use of animals - the three Rs at the centre of the animal activists' arguments - in experimental procedures is incorporated into the legislation.

The third component which is inextricably linked into these issues is the question of the scientific rationale for the work. Thus the use of alternatives must be scientifically justified, or harm to human health may occur. Equally, the experimental design must avoid unnecessary distress to the animals. The selection of the experiments and the details of the experimental design must be scientifically justifiable for it to be acceptable.

PARTICULAR CONCERNS

For the proper conduct of toxicology experiments, it is inevitable or even required that some adverse affects are

produced. This is in contrast to the majority of other experimentation, where pain and distress is an unnecessary but unavoidable consequence of the procedures which may even interfere with the outcome of the experiment. For this reason, toxicology was singled out by the animal rights movement for particular attention. The LD50 test and Draize eye and skin irritation tests received particular attention both because of ethical concerns and for scientific reasons. An example of how Zeneca's Central Toxicology Laboratory has dealt with these ethical issues without compromising the scientific rigour of the testing is given in the next paper in this symposium (P.A.Botham, A Pragmatic Approach to the Introduction of In Vitro Techniques into the Safety Assessment of Pesticides)

The LD50 test

Zbinden and Flurey-Roversi (1981) were among the first to question the value of accurate LD50 determinations requiring the use of large numbers of animals, when it's sole purpose was to categorise the chemical's acute toxicity. Various new methods have now been introduced by the OECD and others (e.g. the Fixed Dose Procedure proposed by the British Toxicology Society) which overcome many of the scientific and ethical concerns.

The Draize skin and eye irritation tests

Concerns arose from the need to produce irritation or corrosion which on occasions could be very severe. Modifications to the methods by, for example, the use of hierarchical testing strategies which start with a consideration of physico-chemical properties and use in vitro methods, have reduced the ethical concerns without compromising the scientific basis or the practical benefits of the results.

Conflict between national animal welfare legislation and regulatory requirements

There is occasionally a conflict between the guidance given under, for example, the UK 1986 Act and the EPA FIFRA requirements for testing. The UK Home Office Guidance on Eye Irritation Tests requires that any animal showing maximal (severe) effects in the OECD Guideline 405 Tests must be withdrawn from the study and killed humanely. In contrast, EPA may require that an observation period of 14 days to provide information on recovery.

Conflict in requirements of this type place companies in a difficult position. It is easy to carry out these experiments to EPA standards in the USA, but this presents a dilemma to those who are genuinely concerned about animal welfare. Is it appropriate to carry out a procedure, which is illegal in the UK, in another country which has lower standards in its animal welfare legislation? Is it sensible to write guidelines which have the effect of reducing the protection given to animals by virtue of exporting the problem to other countries? At the practical level these problems can often be overcome with careful thought and discussion between the parties concerned.

Harmonisation of guidelines

As the objective of toxicology testing is to assess the likely adverse effects to humans and provide information for risk assessment, it should be possible to use identical methods in the different countries. This objective has not yet been achieved for pesticides, although there has been much progress through the auspices of the OECD Chemicals Programme. Retesting merely because there is a requirement for a slightly different protocol has both ethical and cost implications . There is room for further harmonisation and the OECD and International Programme for Chemical Safety of the WHO have an important role to play.

This example illustrates that failure to achieve scientific consensus has the potential to create an ethical problem for those carrying out the necessary animal experiments.

Existing Chemicals

The re-registration of many pesticides is now required in order to bring the regulatory toxicology studies up to modern standards. Re-testing solely because there is a requirement in the regulation should be challenged on ethical grounds and good scientific rationale should be the only justification for retesting.

New guidelines

The last few years have seen the introduction of new testing requirements for neurotoxicity and immunotoxicity. Regulations require a new protocol in addition to the existing toxicity tests. This will provide data specifically designed to inform risk assessment for the particular end-point. However it is possible to modify the existing methods to identify chemicals with the particular toxic properties. Only positive compounds need then be tested in more specific methods. This alternative approach (refinement of the method and reduction of the number of animals) has a better moral justification through limiting the number of animals used without compromising the quality of the risk assessment.

Dose setting

The critical importance of dose setting in the design of toxicity tests is well known to all toxicologists. If inappropriate doses are selected, the study may well have to be repeated. This is of particular concern for setting the highest dose in carcinogenicity studies and the lowest dose level in all studies for the determination of the No-Observed -Effect-Level.

Maximum tolerated dose (MTD)

It is a regulatory requirement that the highest dose used in carcinogenicity studies should be the MTD - usually defined as a dose that will reduce the body weight by no more than 10%. The reason advanced for this is that relatively small groups of animals are used which are surrogates for large numbers of humans.

It is argued that giving a dose which is as high as can be tolerated partly overcomes this problem by increasing the chances of identifying a carcinogenic effect. However, there are other factors to take into account, particularly that very high doses can compromise the physiology of the animal, providing misleading information for human hazard assessment. If the toxicology study does not provide the information suitable for risk assessment, the conduct of the experiment is not morally justified.

No Observed Effect Level (NOEL)

The normal risk assessment method used for agrochemicals requires the definition of a NOEL. It is not uncommon for studies to fail to define a NOEL, and repeat studies are then required. There is an alternative approach which involves calculating the "bench mark dose" or the dose which is predicted to produce a particular response equivalent to a NOEL. In this way information of equal value can be calculated form an existing experiment thus avoiding a repeat. The use of bench mark doses provides a ethically more acceptable means of interpreting toxicity studies.

JUSTIFICATION OF BENEFIT

The usual justification given for regulatory toxicity testing is that the regulations require the testing. There are signs that more justification may in future be required. The Regulatory Toxicity Sub-Committee of the UK Animals Procedure Committee (responsible for monitoring the implementation of the law in the UK) has recently proposed that guidelines by which the benefits of developing substances of various sorts may be assessed should be produced. This, if implemented, has far reaching implications, as it would require some form of benefit analysis for the registered product at an early stage of development. It does however illustrate that ethical concerns continue to impinge on regulatory toxicology.

CONCLUSION

The use of animals for safety evaluation of agrochemicals is the only acceptable way of obtaining information for human hazard assessment. The benefits of developing safer and more effective pesticides to boost food production in a world which is haunted by starvation is self evident. There is a legal and moral responsibility to minimise the pain and distress to which animals are subjected. Scientific judgement is required to justify each study and protocol. There is much to be done to change the regulatory environment so that high quality data for risk assessment is available while the ethical standards in the conduct of toxicology studies is enhanced.

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A PRAGMATIC APPROACH TO THE INTRODUCTION OF *IN VITRO* TECHNIQUES INTO THE SAFETY ASSESSMENT OF PESTICIDES

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ABSTRACT

The term "alternative" has been adopted in toxicology testing to include any method which offers the same or better information on the toxic hazards of a material and which reduces or replaces the use of animals or refines the procedures used to minimise pain and distress. The introduction of *in vitro* alternatives, such as cell or tissue culture methods, has been restricted mainly to the area of genotoxicity where tests such as the Ames test and *in vitro* cytogenetic assays have become widely accepted. In other areas of toxicology, the endpoints measured can be highly complex, involving effects on several organs or homeostatic systems and *in vitro* tests offer few opportunities at present. However, in the case of local toxicity, specifically ocular and dermal irritation, considerable progress has been made and *in vitro* methods are now used as pre-screens, allowing reduction, refinement and replacement of the Draize skin and eye irritation tests.

INTRODUCTION

The number of animals used in scientific experiments in Britain declined each year from 1976, when over 5 million were used, to 1990, when the figure stabilised at about 3 million. In 1992, toxicity tests accounted for 18% of all animal procedures conducted in this country; 3% were required by legislation affecting agricultural chemicals. More detailed figures can be found in two recent publications (Parliamentary Office of Science and Technology, 1992; Home Office, 1994). Accurate figures are not available for many other countries, but a recent report suggests a similar pattern of usage in several other member states of the European Union (CEC, 1994).

The current UK legislation which regulates the use of animals in experiments is the Animals (Scientific Procedures) Act (1986), which is also the means of complying with the EC Directive 86/609/EEC on the protection of animals used in experiments. The Act makes it an offence to carry out any scientific "procedure" on an animal except under licence. Section 5(5) of the Act requires applicants for project licences to give "adequate consideration to the feasibility of achieving the purpose of the programme ..... by means not involving the use of protected animals." Similarly, Article 7(2) of the EC Directive states that "an experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought not entailing the use of an animal is reasonably and practicably available." In other words, it is encumbent on scientists to use alternatives to the use of animals whenever this is scientifically justifiable. Before discussing the area of alternatives to animals in toxicology, the current legal requirements for safety testing, specifically for pesticides, will be reviewed.

SAFETY ASSESSMENT OF PESTICIDES - THE LEGAL FRAMEWORK

In the UK, the primary legislation regulating the use of pesticides is the Food and Environmental Protection Act (1985) and the subsequent Control of Pesticide Regulations (1986). The regulations are in the process of being amended to bring them into line with the new EC Pesticides Directive (91/414/EEC). The vast majority of the safety evaluation process required by this legislation requires the use of experimental animals. The same is true for legislation elsewhere in the world, such as the Pesticide Assessment Guidelines published by the US Environmental Protection Agency (1984) and the Testing Guidelines for the Evaluation of Safety of Agricultural Chemicals published by the Japanese Ministry of Agriculture, Forestry and Fisheries (1985).

Although there are some differences between the requirements of the various regulatory authorities, in essence they are very similar. With the exception of certain genetic toxicity tests (e.g. the Ames test for mutagenicity), all require the use of animals. The general principle of this animal testing is to expose normal, healthy animals to relatively high doses of a chemical to reveal its potential toxicity, as seen in changes in the function or behaviour of the animal or in damage to tissues and organs as detected by haematology, clinical chemistry and gross- or histo-pathology. This information is then used, together with estimates of potential exposure of man, other animals or the environment, to assess the risk associated with the manufacture or use of the chemical.

The majority of scientists and regulators involved in the safety assessment of chemicals such as pesticides believe that the use of sentient animals is normally the only way to gain the best possible understanding of the potential hazards of a substance; toxicity is often expressed in behavioural changes or in multiple organs or tissues or occurs only when a substance is metabolised to a more potent chemical species; conversely, a substance may be poorly absorbed across the gut or skin or may be detoxified by the liver before it can cause significant harm.

Nevertheless, most scientists and regulators involved in toxicity testing are also committed to the development of alternatives to animals. Furthermore, given properly validated scientific data, endorsed by a majority of experts in the relevant field, regulators will change their requirements for animal testing, as has happened in the area of mutagenicity testing where, for example, the Ames and *in vitro* cytogenetic assays are now well-established in test guidelines internationally.

WHAT ARE ALTERNATIVES?

In toxicology testing, the term "alternative" has come to mean not only any procedure which can replace completely the need for animal experiments but also any procedure which can reduce the number of animals required, or diminish the amount of pain or distress suffered (Smyth, 1978). It thus includes all of the "Three R's" proposed originally by Russell and Burch (1959) - reduction, refinement and replacement. Thus, reduction in animal use can be achieved by means of improved experimental design, by multiple use of animals, by the sharing of data between organisations and by the harmonisation of the various international test guidelines. Refinement can include the use of strategies to reduce pain (e.g. the use of anaesthetic), the introduction of tests which use non-lethal endpoints and the use of non-

invasive methods for detecting tissue and organ damage. Replacement of animals can be achieved by the use of cell or tissue culture (*in vitro* techniques), the use of lower order (non-protected) species such as bacteria, plants or insects or early developmental forms of protected species, computer modelling and human studies.

When the definition of "alternative" is widened to accommodate the "Three R's", many examples can be found where progress in animal welfare has been made in the area of safety assessment. In the remainder of this paper, however, emphasis will be placed on the current status of the introduction of *in vitro* methods as "alternatives". As the title of the paper suggests, the approach taken is an essentially pragmatic one, viewed through the eyes of a practising industrial toxicologist whose primary aim is to safeguard human health.

IN VITRO ALTERNATIVES

As has already been mentioned, it is in the area of genotoxicity testing that the greatest progress has been made in the development and validation of *in vitro* alternatives. A package of *in vitro* studies, which includes (a) a bacterial mutation test, (b) a test for chromosome aberrations and (c) a test for mammalian cell gene mutations, is now accepted by most regulatory agencies in the world involved in pesticide registration. If any of these tests are positive, short-term *in vivo* assays would normally then be required. Whilst for a pesticide the results of these *in vitro* and *in vivo* assays do not yet preclude the conduct of animal carcinogenicity studies in both the rat and mouse, they do give a very good indication of the likely outcome of these two-year studies. Thus, a compound which gives negative results in the package of *in vitro* assays is highly unlikely to cause genotoxic carcinogenesis in either bioassay and hence development of the compound, with its significant associated costs, can proceed with more confidence. Conversely, a compound which is positive *in vitro* and confirmed as genotoxic in the short-term *in vivo* assays, may cause cancer by virtue of its genotoxicity. The results of these short-term tests thus give an option to discontinue the further development of such a compound, before significant costs have been incurred. The relative speed and cost-effectiveness of the *in vitro* tests also allows them to be used in this situation to select alternative compounds for development with attenuated genotoxic activity.

For many other toxicological tests, particularly those used to detect toxicity induced by repeated dosing, the endpoints are complex and can involve effects on several organs or homeostatic systems in the experimental animal. In many cases, the exact mechanism by which these toxicological endpoints is induced is not known or poorly understood. Given the present state of knowledge, therefore, it is perhaps not surprising that *in vitro* tests have not yet become accepted as alternatives in areas other than genotoxicity. In fact, it is perhaps unlikely that any *in vitro* system or even combination of *in vitro* tests is capable of modelling the results from the multiple organs, cell types and biological molecules that comprise the whole organism.

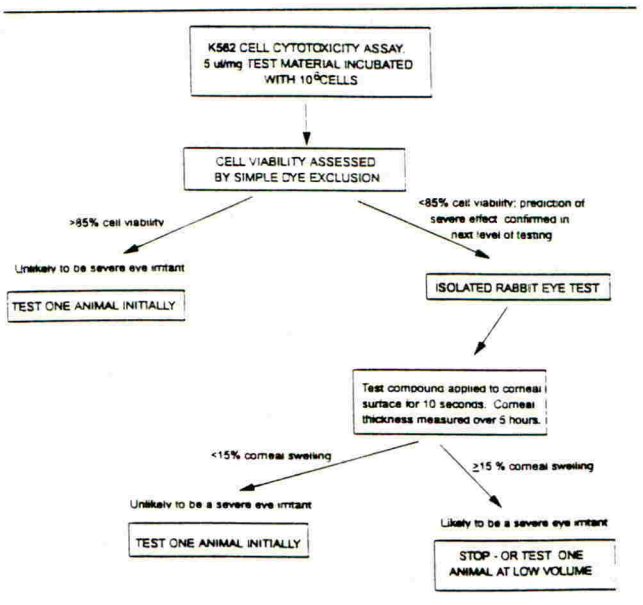
However, the probability of success of an *in vitro* test being able to predict local effects (such as irritation) following application of a chemical to a specific tissue, such as the eye or skin, is reasonably high due to the relative simplicity of the biological endpoint. It is not surprising, therefore, that there has been considerable activity in this area over the last few years.

IN VITRO ALTERNATIVES FOR THE ASSESSMENT OF OCULAR IRRITANCY

Traditionally, the potential of chemicals to cause ocular irritancy is determined in the albino rabbit (the Draize eye test, Draize, 1944). Test material is applied into the conjunctival sac and effects on the cornea, conjunctiva and iris are assessed over a standard time period. This test is particularly emotive, however, and this has accelerated the development of several new approaches to eye irritation testing which aim to both reduce the numbers of animals involved and refine the experimental protocols used. Examples include a wide range of *in vitro* cell cytotoxicity models (Shopsis *et al.*, 1985), *ex vivo* techniques such as the chick chorioallantoic membrane test (Leighton *et al.*, 1985) and the enucleated rabbit eye test (Burton *et al.*, 1981). More recently these techniques have been supplemented by a number of more specialised approaches such as the use of macro-molecular matrices (EYTEX), the silicon microphysiometer (both reviewed by Bruner, 1992) and red blood cell haemolysis (Lewis *et al.*, 1993; Pape *et al.*, 1987).

The use of test batteries, involving complementary procedures varying in endpoint selection and complexity, has also been proposed (Balls *et al.*, 1991). One such battery has been developed in this Laboratory (Figure 1). The battery consists firstly of a rapid cytotoxicity assay to identify chemicals likely to be severe ocular irritants. In this assay, utilising the K562 cell line (Lozzio and Lozzio, 1985), a reduction in cell viability is used as the indicator of ocular damage. Those chemicals predicted to be severe irritants proceed for confirmation to the second tier of testing, the isolated rabbit eye test (Burton *et al.*, 1981), in which changes in the thickness of the cornea are measured as an indication of damage caused by exposure to a test material.

FIGURE 1. CTL stepwise approach to eye irritancy testing.



Data obtained using the tiered *in vitro* test battery are used to guide any subsequent *in vivo* test, as shown in Figure 1. By predicting as accurately as possible those materials which are likely to be severe eye irritants *in vivo*, the number of animals used can be reduced (e.g. by testing initially in a single rabbit) and the severity of the response can be lowered (e.g. by applying the material at a lower volume or at a suitable dilution); if a severe response is seen, no further animals are required.

A 'severe' *in vivo* effect is defined as a Grade 6 or above on the Kay and Calandra scale (Kay and Calandra, 1962), which is an internationally-recognised scoring system used to interpret and rank the results of *in vivo* rabbit eye tests. Of 189 chemicals tested, both *in vivo* and *in vitro*, the majority of severe eye irritants (19 of a total of 23) were correctly predicted *in vitro*. The test battery has, therefore, proved to be 83% sensitive (the ability to correctly identify severe eye irritants). A high specificity (85%) i.e. the ability to correctly identify those materials which are less than severely irritating, has also been observed (Lewis *et al.*, 1994).

A direct comparison of the performance of the *in vitro* testing battery can be made with the ability of *in vivo* skin irritation data to predict ocular irritancy, a pre-screen recommended in the OECD guideline on eye irritation (OECD, 1987). The ability of the results of skin irritation tests to correctly predict severe ocular irritancy was much poorer. Only 13 of a total of 57 chemicals having severe effects in the eye were correctly predicted from the severe effects they had caused on the skin, giving a sensitivity of only 23%.

AN *IN VITRO* ALTERNATIVE FOR THE ASSESSMENT OF SKIN CORROSIVITY

The assessment of skin toxicity is also a vital part of the standard battery of toxicological tests carried out on chemicals. The primary response of the skin to chemical contact may be a reversible inflammatory irritation characterised by redness and swelling, or an irreversible change involving cell or tissue death (necrosis) and scar formation. This latter phenomenon is termed a corrosive response. Traditionally, the potential of chemicals to cause primary skin effects is determined by the Draize test (Draize, 1944). Test material is applied directly to a shaved area of skin for up to 4 hours and irritation or skin corrosion is assessed subjectively over a standard time period.

A corrosive response results from a physico-chemical interaction between chemical and skin tissue and this event usually occurs within 24 hours after skin contact. In this Laboratory, an *in vitro* model system has been developed for this response. The model is based on the loss of barrier function of the outermost layer of the skin, the stratum corneum, as a sequel to the directly damaging effects of corrosive chemicals. This event is detected by measuring the reduction in electrical resistance across isolated rat skin after treatment of the tissue *in vitro* with corrosive materials. The rat skin used in this test is obtained from the dorsal pelts of young animals. Many skin preparations can potentially be obtained from the pelt of a single animal.

Initial validations of the technique (Oliver *et al.*, 1986, 1988) and subsequent refinements of the method (Barlow *et al.*, 1991; Botham *et al.*, 1992) together with interlaboratory validation (Botham *et al.*, 1992) have confirmed its utility and reproducibility. A recent evaluation of the test (Lewis RW, unpublished results) in predicting the corrosive

potential of 338 diverse chemicals showed that this assay correctly predicted 90% (19 of a total of 21) of skin corrosives tested. This high sensitivity was combined with a high specificity (94%), the ability to correctly identify non corrosive materials (299 out of a total of 317).

The test battery for eye irritancy and the *in vitro* corrosivity test are rapid, technically simple, reproducible and objective techniques that are now integrated into assessments of skin and ocular toxicity at CTL as one part of a testing cascade allowing decisions to be made on the subsequent use of live animals:-

1. If a positive result is obtained, then in some circumstances (for certain classes of chemical and for hazard handling and safety assessment within the company), animal tests are not conducted (**REPLACING** the use of animals completely).

2. If confirmatory animal tests are required, then tests can be designed to **REDUCE** the number of animals used (often reducing the number of animals tested from 6 animals to a single animal) and to **REFINE** experiments by shortening the duration of application to the skin or by applying smaller volumes or dilutions of test chemical to the eye, so that possible pain and distress is kept to a minimum.

THE FUTURE

At the moment, developments in the area of *in vitro* alternative safety tests are largely confined to areas where the biological endpoint is specific and defined, where a coherent *in vivo* mechanism can be identified and where the effects of a single (acute) dose or application are studied, as exemplified above by skin corrosion and eye irritation testing.

The future evolution of *in vitro* techniques in safety assessment rests on a number of essentials. The first is a commitment to understand the mechanism of toxicity leading to a given observed endpoint. Mechanistic studies in toxicology are in themselves areas of opportunity for refining, reducing and replacing the use of animals, in that such investigations often consider specific target organ or target cell toxicity which may be studied in isolation. Armed with data on the mechanism of toxicity, the toxicologist is in a position of strength from which to design and develop alternative test methods.

The second essential is high quality evaluation and validation of alternative tests to ensure both scientific and 'legal' (regulatory) acceptance. The 'gold standard' against which alternative procedures are judged are the results of standard animal toxicity tests. Hence, accurate databases of *in vivo* test results need to be collated, published and used in the evaluation and validation processes (Purchase, 1990; Bagley *et al.*, 1992). An added advantage of developing reference chemical databanks of this type is that this activity should not involve the use of a significant number of additional experimental animals simply to provide data for comparison purposes. The development and use of such databases is essential to the proper standardisation, intra- and inter-laboratory evaluation and ultimately the 'validation' (the process whereby relevance and reliability are established) of alternative tests.

CONCLUSION

The development and validation of *in vitro* alternative methods for predicting the toxic hazard of materials is a legitimate exercise both on scientific and ethical grounds. However, the expectation of success for these methods is often seen simply in terms of their immediate effect on the reduction, refinement and replacement of the use of laboratory animals. Whilst for certain areas of toxicology, notably the assessment of skin and eye irritation, this is a realistic short-term goal, for others the major contribution of *in vitro* methods may in the near future lie in the investigation of toxicological mechanisms following the discovery of adverse findings in standard animal toxicity tests. Any longer-term goal to replace entirely the use of animals in toxicology may not be entirely misguided, but will certainly require significant advances in scientific understanding and the development of appropriate technology.

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THE RELEVANCE OF METABOLISM IN THE SAFETY ASSESSMENT OF AGROCHEMICALS

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ABSTRACT

Toxicology studies using laboratory animals provide the basis for establishing the toxicological potential of agrochemicals. While these studies evaluate toxic effects and relate them to administered doses resulting in the definition of a no observable effect level metabolism studies are performed to investigate the fate of the test compound in the animal namely absorption, tissue distribution, rates and routes of excretion and the biotransformation pathways. The occurrence of toxic effects is better correlated with systemic exposure, consisting of the test compound and its metabolites than administered dose but often the way metabolism studies are currently performed only allows a qualitative estimate of this parameter in toxicology studies. Since the quantitative systemic exposure to a pesticide and its metabolites can vary considerably according to the dose administration procedure it would be preferable to have more precise information on systemic exposure of animals used in toxicology studies.

Species differences in metabolism are well established which may have a direct influence on the toxicological profile and in the absence of information a rational extrapolation from laboratory animals to man is not possible. The conduct of controlled low dose metabolism studies in human volunteers can provide valuable data which will allow a comparison with laboratory animals and also selection of a marker compound that could be used to monitor exposure of different population groups.

INTRODUCTION

The goal in the safety evaluation of pesticides is to evaluate consumer and worker exposure and subsequently perform a risk assessment. The process begins with an investigation of toxicity using laboratory animals and it is from these studies that toxicological potential is determined and no observable effect and adverse effect dose levels established. Here there is a fundamental problem that toxicity is evaluated in laboratory animals such as rats and dogs, often at maximum tolerated dose levels and attempts made to extrapolate the results of these studies to human exposure. This regulatory toxicology process has become established over many years and although the scientific community involved in the process increasingly recognise the unscientific approach old habits die hard and there is a general reticence to question the approach which one can argue has been tried and tested. Companies developing compounds know they have to satisfy the regulatory requirements of government agencies and these authorities may have to defend their actions to the general public if subsequent problems occur. Any change needs to be a joint effort of all interested parties for whom there should be a common goal.

Toxicological effects are related to the extent of exposure of organs/tissues to the test compound and/or its metabolites but dose levels do not necessarily provide a good index of this exposure. Firstly, there is a dependence on the extent of absorption which may be dose-dependent and route dependent (eg. oral vs dermal). The dose formulation may also have a profound effect on the rate and extent of absorption (Hawkins, 1988). Unfortunately, the word exposure has different meanings and according to the U.S. E.P.A exposure is the amount of material available for absorption at points of entry into the body such as skin, lungs and the digestive tract and is therefore equivalent to the dose. For a compound that is not absorbed after ingestion the gastrointestinal tract is exposed to the total dose but there is no exposure of other tissues/organs. The term systemic exposure can be used to relate to the amount of material absorbed into the body and the blood/tissue concentration time profiles for the test compound and its metabolites.

The *in vivo* toxicology profile is also often determined by the biotransformation processes which occur and their rate and there can be large species differences in these processes. Toxicity may be due to the intrinsic chemical reactivity of the test compound or the formation of reactive intermediates by biotransformation (Hawkins, 1988). Processes exist to protect against toxic intermediates but as the concentration of these increase detoxification pathways may become saturated leading to the onset of toxicity above a threshold dose level. The question is how can we use data generated by appropriate investigations of metabolism and kinetics for a more rational approach to safety evaluation.

RELATIONSHIPS OF METABOLISM AND TOXICOLOGY STUDIES

A key aspect forming part of the risk assessment process concerns an understanding of differences in metabolism and mechanisms of toxicity in animals. The extent to which this can be achieved depends on the test compound. Very sophisticated detailed investigations can be performed however, in the real world unlimited time and resources cannot be allocated to this activity. While the scientific literature contains examples for existing compounds this has usually been carried out retrospectively when considerable background knowledge already existed and for compounds with a less complicated mechanistic toxicity profile. We need to address what can be achieved in a reasonable timeframe for new or existing compounds where little is known.

There are current regulatory requirements for rat metabolism studies but these studies are often carried out in isolation from toxicology studies and it is clear that frequently the potential value of these investigations is not fully exploited. Given that there are relationships between metabolism and observed toxicity there is a considerable difference in some key aspects concerning the conduct of the respective investigations (Table 1).

TABLE 1. A comparison of the design of and data from rat metabolism and toxicology studies

	Metabolism	Toxicology
Dose formulation	Solution/suspension in water/corn oil/polyethylene glycol/aq. carboxymethyl cellulose	Dietary incorporation
Administration	Gavage (bolus dose)	*Ad libitum* in diet
Dose levels	One or two (mg/kg bodyweight)	Three based on dietary ppm
Duration	Usually single doses	Daily for duration of study
Absorption	Quantifiable	Unknown
Systemic exposure	Blood/tissue concentrations and half-lives determinable	Unknown
Relationship of dose level to absorption and systemic exposure	Can be assessed	Unknown

It is evident that given the differences in how metabolism and toxicology studies are conducted it is difficult to relate the two types of investigation. Since dose quantification and accuracy is of prime importance in metabolism studies with the need to obtain high accountabilities of the administered dose, dose formulations are devised to facilitate this requirement. Generally this means that doses are formulated in way that optimises absorption such as small particle size suspensions or in solution. The type of dose vehicle has a profound effect on local exposure of the gastrointestinal tract and on the blood and tissue concentration-time profile of the test compound. It has been estimated that for a given daily dose the maximum concentrations in the stomach vary in the ratio 10 (gavage) : 2 (diet) : 1 (drinking water) (Clark & Smith, 1984). Lipid vehicles can reduce the rate of gastric emptying and in some cases diminish the rate of absorption. A high fat intake can also have physiological effects on cell membranes and oxidative enzymes. Corn oil tends to delay absorption and at high dose levels can reduce liver exposure to the test compound below that at which metabolism is saturated (Angelo *et al*, 1986). Corn oil may also provide a protective effect on a chemical by preventing its decomposition at the pH's encountered in the aqueous environment of the gastrointestinal tract.

Metabolism studies as currently performed will provide some basic information about the fate of the compound such as distribution indicating target tissues, rates and routes of excretion, the nature and identity of metabolites and the saturation of any of these processes with increasing dose level. However, the rates of different processes play a vital role in the overall profile of systemic exposure to the compound and its metabolites. Currently it is not possible to use the knowledge from metabolism studies to estimate the systemic exposure of animals in toxicology studies. There is usually no direct information on absorption and ...

... the established approach has been to keep increasing dose levels until effects are observed which then proves that some compound must be absorbed. This is one reason for the establishment of the maximum tolerated dose concept in toxicology. There would seem to be a need to try and improve this situation. In rodent toxicology studies effects can be both species and sex-specific. For some compounds when equivocal results have been obtained studies have often been repeated one or more times often with different results. We have no basis for explaining these paradoxical results but if we had information on the systemic exposure of the animals it might provide some answers and would certainly be one way of relating one study to another.

Mammalian metabolism in agrochemical development does have other important uses, a major one being to establish that where plant/crop metabolites are encountered as significant residues these also occur as rat metabolites ensuring by autoexposure that their toxicity is evaluated as part of the parent compound toxicity studies. It is however important to design metabolism studies that meet all the desirable objectives and this may require a modification of the current approach.

SPECIES DIFFERENCES

The objective after performing metabolism and toxicity studies in laboratory animals is to use the data to make a risk assessment for human exposure. It is well known that species differences exist and that toxicologically man is not a large rat. This is nominally incorporated into the risk assessment process by assuming that man is the most sensitive species and using an additional tenfold safety factor to the no observable effect level for a possible interspecies difference. This may be totally inappropriate and it would be preferable to have a scientific basis for making interspecies comparison. There are some well established differences between rat and man which can have very significant toxicological implications (Table 2).

TABLE 2. Differences of potential toxicological significance between rat and man

	Relative amount/activity	
Parameter	Rat	Man
Flora in stomach, proximal small intestine	Numerous	Little or none
Intestinal β-glucuronidase	Very high	Low
Skin stratum corneum/vasculature	Thin	Thick
Plasma protein binding	Lower	Higher
Biliary excretion	High	Low
Urinary excretion	Lower	Higher
Conjugations		
Glucuronidation	High	Low
Sulphation	Low	High
Acetylation	Effective	Effective or slow
Amino acid with aryl acetic acids	Glycine	Glutamine
Quaternary N-glucuronides	Low	High

The more extensive gut flora in the rat and the greater excretion of compounds in the bile which returns material to the gastrointestinal tract, means that some rat-specific metabolites can be formed and subsequently absorbed into the systemic circulation. The process of enterohepatic circulation can also maintain the body burden of compound-related material prolonging the clearance of some metabolites.

The anaerobic environment of the gut means that reductive processes can predominate. An example is the reduction of a nitroaromatic to an arylamine which can occur after excretion of an initial nitroaromatic metabolite in bile. Given the toxic potential of arylamines which can be absorbed after formation in the gut this could lead to a substantially greater toxicological hazard in rat compared to man.

Although in general the same type of metabolic processes occur in laboratory animals and man there are often species differences for specific compounds. Some pathways are indicative of the formation of reactive intermediates such as glutathione conjugation resulting in the ultimate excretion of mercapturic acid conjugates in urine. The electrophilic intermediates formed may be associated with toxicological end points and measurement of a mercapturic acid metabolite in urine is one method of assessing species differences in this pathway. In some cases man has evolved a more efficient way of metabolising compounds one example being formation of quaternary N-glucuronides. For some compounds this represents a major pathway in man while it is minor or non-existent in laboratory animals. It has the advantage for some molecules that no phase I metabolism is required to introduce a functional group for conjugation. The levels of enzyme activities in tissues can also be an important factor since the higher the concentrations of toxic metabolites formed in tissues the greater the potential to cause tissue damage. The relative activities for some liver mono-oxygenases indicate that this is much lower in man compared to the rat while glucuronyl transferases are more similar (Table 3). One oxidative process is formation of epoxides which can have toxicological potential. Hydrolysis is one detoxification pathway for these intermediates and the activity for a dieldrin analogue is considerably higher in man compared to rat (Table 3). Hence, in man the activity for formation of a potentially toxic metabolite (oxygenase activity) may be less and the detoxification higher.

TABLE 3. Relative microsomal enzyme activities adjusted for liver weight/body weight ratio

	Mono-oxygenase (mean for several substrates)	Glucuronyl transferase	Epoxide hydrolase (HEOM*)
Man	0.13	0.66	15
Rhesus monkey ♂	0.54	1.1	
Squirrel monkey ♂	0.24	2.4	
Rat ♂	1.0	1.0	1.0
Rat ♀	2.6	1.3	2.5
Mouse ♂	1.8	0.44	0.33

*Analogue of dieldrin

Dermal exposure is particularly important for agrochemicals and in considering the hazards resulting from this route the rate and extent of dermal absorption is a key parameter. Percutaneous absorption has very different characteristics compared to oral absorption such as large species differences, greater variation between compounds and is more readily saturable. The amount of material absorbed will be dependant on the application rate ($\mu g/cm^2$) and the total area exposed. Information on dermal absorption assumes great importance with respect to extrapolation to man since dermal absorption in laboratory animals such as the rat is much more extensive than in man (Wester & Maibach, 1985) (Table 4). Therefore the experimental measurement of dermal absorption in man can be extremely valuable.

TABLE 4. Species comparison of *in vivo* dermal absorption expressed as percentage applied dose (from Wester & Maibach, 1986)

	Rat	Rabbit	Pig	Man
Haloprogin	95.8	113	19.7	11.0
Cortisone	24.7	30.3	4.1	3.4
DDT	-	46.3	43.4	10.4
Lindane	-	51.2	37.6	9.3
Parathion	-	97.5	14.5	9.7
Malathion	-	64.6	15.5	8.2

HUMAN STUDIES

One of the most difficult aspects of toxicological risk assessment is extrapolation to man. A knowledge of the comparative absorption, metabolism and excretion in laboratory animals and man can provide valuable data to assist with this extrapolation. The most relevant type of study is a controlled low dose metabolism/kinetic study which will allow a comparison with laboratory animals and also selection of a marker compound that could be subsequently used to monitor exposure of different population groups.

There has been a general reticence to perform human volunteer metabolism studies with agrochemicals primarily due to the perceived ethical problem. These studies form an integral part of drug development programmes and procedures for ethical review are established. Human volunteers participate in a study for the purpose of generating valuable scientific data and do not receive a direct benefit. It is therefore immaterial to them whether the test compound is intended as a drug or agrochemical provided equivalent considerations have been given to the safety of the proposed dose administration in the study. In many cases agrochemicals belong to structural classes which have counterparts as human drugs (eg. triazole fungicides). There is an increasing awareness of the value of these studies which is reinforced when data for specific compounds is generated. With the existence of specialist clinical pharmacology units experienced in the organisation and conduct of human ...

... volunteer studies there is no reason in the UK why appropriate human metabolism/kinetic studies with agrochemicals cannot be carried out. The data obtained enhances the scientific value of laboratory animal toxicology studies for risk assessment which strengthens the justification for the use of animals in the safety evaluation process.

STRATEGIC APPROACH

The case has been made for the value of performing metabolism studies but the more difficult aspect is designing the most appropriate experiments. It is not possible nor desirable to define precisely the details of an investigative programme since some aspects will be compound-dependent. However, an outline of the different types of investigation that should be considered are listed below.

1. Conduct single oral dose experiments in laboratory animals at a minimum of two dose levels to establish intrinsic data on absorption, distribution and excretion and dose proportionality.

2. Investigate pathways of biotransformation and establish whether saturation of processes occur with increasing dose.

3. Use data to make rational selection of dose levels in toxicology studies and/or evaluate systemic exposure of animals dosed under the conditions of toxicology studies.

4. Evaluate mechanisms of observed toxicity if feasible and where appropriate. Use *in vitro* studies to evaluate species differences in metabolism and toxicity including man.

5. Consider human volunteer/worker exposure studies to investigate absorption, metabolism and excretion in order to evaluate human systemic exposure in comparison with that of laboratory animals in toxicity studies. Generate data to allow selection of a marker compound as an index of human exposure.

6. Use experimental data generated and scientific knowledge available on the toxicology, metabolism and species-specific activities of structurally-related compounds to provide a rational risk assessment.

CONCLUSIONS

The techniques and technology now available for investigating metabolism have advanced considerably over the last few years. It is now possible to obtain higher quality information in a shorter time which is a major advance provided we design the appropriate studies to generate useful data. There is generally a widespread belief that metabolism data is important and valuable but the generation of appropriate data and its use has generally not yet been fully exploited. This can only happen as a joint initiative involving regulatory authorities and industry but there is no doubt it would be of benefit to all including society in general.

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HOW ARE REGULATORY AGENCIES ABLE TO USE THESE MODERN APPROACHES TO TOXICITY TESTING?

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ABSTRACT

Methodology currently used in the UK Pesticides Safety Directorate for risk assessment of consumer and operator exposure is described. Modern approaches to toxicity testing generate data which have not been routinely used in these processes in the past. However, the current framework is such that results of modern tests can be incorporated without changing the basic principle that good science will always contribute towards sound decisions.

INTRODUCTION

The Pesticides Safety Directorate (PSD) is an agency of the Ministry of Agriculture, Fisheries and Food, which leads the UK regulatory system for control of agricultural pesticides. The legal framework is currently derived from the Food and Environment Protection Act (1985) and the Control of Pesticides Regulations (1986). PSD operates the system on behalf of six government departments (The Ministry of Agriculture, Fisheries and Food, The Department of Health, The Department of the Environment, The Department of Employment and the regional offices for Scotland and Wales) and all these ministers are jointly responsible for granting approvals. In granting approvals for specified uses of pesticides, ministers are advised by the independent Advisory Committee on Pesticides. A harmonised European system for pesticide approval within the European Union is now being introduced. It is expected that risk assessment methodologies currently used in the UK will be compatible with the European system.

THE CURRENT SYSTEM

The safety of chemicals intended for use as pesticides is assessed in exactly the same way as other groups of chemicals, which have intentional or unintentional exposure to man, such as drugs, veterinary medicines and food additives.

As a first step in any explanation it is often beneficial to sort out the definitions used. In this case it is important to recognise the difference between hazard, the potential to cause an adverse effect, and risk, the expected frequency of an undesirable effect arising from exposure to a chemical. It follows that hazard is an intrinsic property of a chemical, while risk incorporates exposure. A hazardous chemical can be acceptably safe, provided exposure is controlled. In the case of pesticides, workers applying a product to a crop might wear protective clothing, or for consumers it can be the case that application of a pesticide to a crop leaves no residue in that crop. The basic principle of pesticide control is that the permitted

uses, specified on the approved label, are acceptable. Regulatory approval is backed up by enforcement (including training), and post marketing surveillance (including usage surveys, residue monitoring and adverse effect monitoring).

The first steps in risk evaluation involve hazard identification and dose response assessment. These two steps are the basis of toxicology. The toxicological screening process begins with acute single dose testing, progressing through short-term repeat dose studies and studies of absorption, distribution metabolism and excretion, to life span studies in animals and includes studies of special functions such as reproduction. With regard to areas such as immunotoxicology and neurotoxicity, it is accepted that these general toxicology studies give some indication of an effect which would be further investigated in special studies. This approach has gained international acceptance through the Organisation for Economic Co-operation and Development, which operates an international harmonisation programme for toxicity testing protocols.

The results of these toxicity tests give us details about the toxic hazards posed by the chemical along with information about the mechanism of action. In quantitative terms we are able to define no adverse effect levels. These no adverse effect levels are used in various risk evaluation processes.

In the case of pesticides there are mainly two potential routes of human exposure. Consumers of agricultural produce derived from treated crops may be exposed to residues in food and agricultural workers may be exposed to pesticides in their workplace. These exposures are dealt with in different ways. Consumer exposure is compared with an acceptable daily intake (ADI). This is generally defined as the dose (in milligrammes of compound per kilogramme body weight) to which a person may be exposed on a daily basis throughout life, without any harmful effect. The ADI is derived from the toxicology data base by looking for the most crucial no effect level. This may be the lowest no effect level, but other considerations, as described later, also effect this choice.

The no effect level, the dose causing no adverse effects in animals, is divided by a safety factor to give the ADI. Although the term safety factor is commonly used, some scientists prefer the term uncertainty factor. This is because the size of the factor has little to do with safety, but is determined by levels of concern and uncertainty. The factor has to account for interspecies and intraspecies extrapolation. There is a body of evidence from which it may be concluded that the difference in sensitivity to chemicals between different species is never greater than 10-fold, and similarly that within a species the difference between a sensitive and a resistant individual is never greater than 10-fold. Thus the conventional safety factor combines these two 10-fold factors, to give an overall 100-fold factor. But this is only a starting point. The factor may be reduced (if, for example, human toxicology data are available) or increased (if, for example, there is uncertainty about the no effect level or concern about serious effects at doses above the no effect level).

It is this combination of no effect level and uncertainty factor which sometimes means that the lowest no effect level is not the most crucial. In some cases a study with a higher no effect level can be used for derivation of a reference dose because the effects seen in that study are such as to require a higher uncertainty factor and hence lead to a lower reference dose. In this process there is no substitute for expert evaluation on a case by case basis.

The outcome of the process is the derivation of an ADI from the toxicology database. In a similar fashion an admissible operator exposure level (AOEL) is derived from the toxicology data. This could be different from the ADI because the duration of operator exposure is different from that of consumers and the route of exposure may be taken into account.

The overall conclusion of the toxicology assessment is the derivation of these two reference doses, the ADI and the AOEL. However, these reference doses, though expressed numerically, do not have a great degree of numerical accuracy. They are derived from no effect levels, which are dose levels in toxicology studies. The dose level is not a continuous variable, but changes in large steps from one treatment level to the next. It is not inconceivable that different studies, with different dose levels, investigating exactly the same material, could conclude with no effect levels and hence reference doses which differ by as much as five fold.

The next stage in the regulatory process involves the examination of the proposed or existant uses of the pesticide product, to see whether the potential exposure levels are permissible. Consumer exposure can be calculated from residues data (supplied as part of the registrants data package) and food consumption data derived from surveys carried out by government. Again, the generation of residues data relates back to the approved label. Relevant data are those which include the maximum application rate and the shortest pre-harvest interval. Operator exposure can be predicted from models based on experience of agricultural work practice or it may be actually measured in field experiments.

Only in cases where predicted exposure is less than the permissible reference values will approval for use be granted.

Behind the assessment of toxicology data and derivation of reference doses there is a simple decision tree approach which begins with some understanding of whether the effects observed in animals are relevant to man. In the absence of detailed information, regulators must err on the side of caution and assume that all responses seen in test animals might also be seen in man.

THE "NEW" DATA

Over the past fifteen to twenty years the conduct of regulatory toxicology has improved dramatically. Although this has occurred at the same time as the introduction of Good Laboratory Practice (GLP) regulations, it is important to remember that GLP has little to do with science. GLP has contributed significantly to the improvement of record keeping and the ability of regulators and investigators to "reconstruct" studies and have increased confidence in the validity of results. Advances in the science of regulatory toxicology have been directed at improving the utility of experimental data in extrapolation from animals to man.

Mechanistic data to describe the processes which occur in animals and enable informed decisions to be made about the possibility of such effects occurring in man, can obviously aid risk assessment. This is the major contribution of the so called "new" toxicology data. The actual data generated and the methods used are often not novel, but the thought process and use of the data represents a great improvement in regulatory toxicology.

Mechanistic data

Direct mechanistic data which demonstrates that effects seen in animal studies could not occur in man is the first obvious example of how specialised data can affect the regulatory process. A number of chemicals produce kidney neoplasia in male rats. Investigative work has revealed that the mechanism for this process involves binding of the chemical to α2-microglobulin. The protein complex accumulates in the kidney tubules, with resultant necrosis and subsequent cell proliferation. Since the protein is specific to male rats, the relevance of the finding for man can be questioned and the neoplasia seen in the animal studies can be disregarded for risk assessment purposes (Swenberg *et al.*, 1989).

Tumorigenicity associated with peroxisome proliferation (a phenomenon to which humans appear to be particularly unresponsive) is another case where results of experiments in rodents probably have little predictive value for risk assessment (UK Department of Health, 1992).

Metabolic saturation

It is well known that the toxicity of many compounds is determined by enzymatic processes of metabolism and elimination and are not due simply to the parent compound itself. Similarly, detoxification of some chemicals or their active metabolites is determined by metabolic processes. These systems of activation, detoxification and elimination are capacity limited and subject to saturation under high dose conditions frequently employed in toxicology studies. High dose specific toxicity can sometimes be attributed to toxic mechanisms which are only seen under conditions of saturated metabolism or elimination. Carefully conducted metabolic studies can be pivotal in determining that high dose phenomena can be disregarded for risk evaluation.

In the case of ortho-phenylphenol (OPP) rats fed high doses developed malignant bladder tumours while lower doses provided no evidence of bladder tumours (Hiraga and Fujii, 1981). Metabolic studies showed that OPP was completely metabolised to sulphate and glucuronide conjugates at low doses, while at higher doses increasing amounts escaped conjugation and were subjected to mixed function oxidase metabolism to yield reactive quinone metabolites (Reitz, *et al.*, 1983, 1984).

Hormonal imbalance

A further example of how mechanistic data can affect the risk evaluation process is in the area of hormonal imbalance. The ethylene-bis-dithiocarbamate (EBDC) fungicides are a class of chemicals which cause thyroid tumours in rat carcinogenicity bioassays. However, the underlying mechanism is one of chronic excess secretion of hormones controlling the endocrine system. A no effect level can be established for this underlying process and a reference dose based on the mechanism of action. Further assurance of safety is provided by the fact that the mechanistic data lead to the conclusion that occasional disturbance of hormone levels outside normal ranges would not immediately lead to tumour induction. Persistent, chronic elevation of hormone levels is required to lead to tumour formation, a situation which would not occur in normal dietary consumer exposure to low levels of pesticide residues (Hill, *et al.*, 1989).

Atrazine is a chloro-triazine herbicide used extensively for broad-leaved weed control. In some rat studies, treatment with atrazine leads to an increased incidence of mammary tumours. Investigative work has revealed that this effect is caused by hormonal imbalance resulting from interference with the regulation of estrogen. Linking this work to knowledge of estrous cycle control in rats and humans has meant that the relevance of the finding in rats for risk assessment in man has been questioned (Wetzel, *et al.*, 1994).

Cell proliferation

It is now generally accepted that chemically induced cell proliferation can lead, in long term toxicology studies, to an erroneous conclusion that the test material is directly causing an increased tumour incidence. This could be because the increased cell proliferation means that more DNA replication is available for spontaneous mutation to occur, or it is also possible that the increased proliferation provides some kind of preferential growth advantage to spontaneously induced cancerous cells. Cell proliferation can be produced by a cytotoxicant, causing cell death followed by regenerative proliferation, or by mitogens which directly induce proliferation associated with organ growth. The important point to remember is that the cell proliferation is an effect that might occur in man, but that the phenomenon can be investigated and a no effect level established.

The carcinogenicity of chloroform is hypothesised to be secondary to the chemically induced cytotoxic response and subsequent cell proliferation (Reitz, *et al.*, 1990), while phenobarbital is generally considered to be a mitogenic carcinogen (McClain, 1990). Knowledge of the mechanisms and conduct of well designed investigative studies can yield data which are crucial in the risk evaluation process.

In vitro techniques

There are many new *in vitro* techniques which have been developed over the past few years. In some cases, such as the *in vitro* or *ex vivo* skin and eye irritation techniques, one of the objects of the new tests is to reduce the use of experimental animals. This is a laudable aim and should continue to be encouraged and supported by regulatory authorities world-wide. However, there will always remain the problem of validation. Well conducted international validation programmes are required before regulatory authorities will accept that new tests are good substitutes for their long experience with established tests. The commercial nature of some of the newer tests tends to hamper this validation process.

There is one area of *in vitro* experimentation which has already proved useful in pesticide regulation in the UK. This concerns the dermal penetration of pesticides. Dermal penetration data can be very important in consideration of operator exposure since, with most application methods, the greatest proportion of operator exposure is via the dermal route. *In vitro* techniques have been developed which use skin sections to investigate the extent and rate of penetration of pesticide formulations through the skin barrier. These techniques have advantages and disadvantages. The major disadvantage is that the skin section lacks the complete processes of blood flow and perspiration. The major advantage is that human skin can be used, whereas *in vivo* studies in man in this area are rare. The state of validation of these *in vitro* techniques is such that regulatory authorities are sometimes reluctant to accept *in vitro* data alone. However, a combination of *in vivo* data in animals and *in vitro* data to compare animal and human skin, can be very useful to regulators.

THE FUTURE

Having reviewed a selection of data types which can be generated by the so called "new" toxicology it is evident that many of these techniques have been used in the past in a retrospective fashion. Unusual or "difficult" results have been obtained in routine toxicology studies and investigative studies have been conducted to explain the results. The current challenge is to move these techniques to the prospective area, to investigate mechanism and metabolism before starting the longer term studies and to use all the available information in setting sensible dose levels. The greater challenge to the regulators is to ensure that this work is required only as and when needed, and not to insist on generation of a fog of gratuitous data. Toxicologists have an obligation to maximise exposure in toxicity studies in order to investigate dose response, but the highest exposure has to remain relevant to the object of the experiment - risk assessment for man.

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