Persistence and management of enhanced carbetamide biodegradation in soil

S J W HOLE*, N C McCLURE† & S B POWLES‡

Department of Applied and Molecular Ecology, University of Adelaide, †School of Biological Sciences, Flinders University, Adelaide, and ‡WA Herbicide Resistance Initiative, Faculty of Agriculture, University of Western Australia, Nedlands, Australia

Received 6 September 2000
Revised version accepted 11 January 2001

Summary

The extent of enhanced degradation of the herbicide carbetamide declined over time after herbicide application was discontinued. The kinetics of carbetamide degradation were determined in the same soil for three consecutive years (1994–96) after single annual applications from 1989 to 1992. The DT_{50} of carbetamide increased from 5.4 d in 1994 to 10.2 d in 1996. However, this was still less than the DT_{50} in previously untreated soil (23–44 d). A most probable number (MPN) assay demonstrated a link between carbetamide degradation rate and the numbers of micro-organisms capable of carbetamide mineralization. Degradation of six other herbicides was assayed in the carbetamide-pretreated and the previously untreated soils. Propham was the only herbicide which degraded more rapidly in the soil with a history of carbetamide application. Rapid degradation of chlorpropham, a herbicide structurally similar to carbetamide and propham, and propyzamide, a herbicide with similar mode of action and weed control spectrum, was not observed. The results suggest that enhanced biodegradation of carbetamide can be managed by less frequent carbetamide application as a part of a herbicide rotation involving compounds which are structurally dissimilar.

Keywords: enhanced biodegradation, carbetamide, soils, pesticide, persistence, Australian soils.

Introduction

Carbetamide ((R)-(–)-1-(ethylcarbamoyl)ethyl phenylcarbamate) is a soil acting herbicide which controls annual grasses and suppresses some annual broad-leaved weeds in a range of dicotyledonous crops. As demonstrated for numerous other soil-applied pesticides (Roeth, 1986; Racke & Coats, 1990), carbetamide has been shown to degrade more rapidly in soil after repeated application (Hole & Powles, 1997). Carbetamide is a relatively slow-acting herbicide, taking several weeks to kill plants, but can be rapidly metabolized in the plant (Desmoras *et al.*, 1967). These properties require continual or repeated uptake of herbicide from soil for effective weed control. If carbetamide is degraded rapidly in soil, there will be reduced availability

Correspondence: S J W Hole, Syngenta, Jealott's Hill Research Centre, Bracknell, Berkshire RG42 6ET, UK.
Tel: (+44) 1344 414918; Fax: (+44) 1344 413677; E-mail: stephen.hole@syngenta.com

to plants, leading to poor herbicidal efficacy. This has been reported to occur after repeated carbetamide application to the same soil (Hole & Powles, 1997).

Strategies for preventing or circumventing enhanced pesticide biodegradation when applied to soil can be classified into either operational or technological (Felsot & Tollefson, 1990). Operational strategies are those which are based on biological principles, whereas technological strategies are those which use pesticide formulation technology or chemical structural changes to obtain acceptable control. Strategies for management of enhanced biodegradation rely on either avoiding or suppressing the component of soil biological activity responsible (Robertson & Alexander, 1994).

Studies concerned with management of enhanced biodegradation have focussed on those pesticides where economic losses have been greatest. For herbicides this has been for thiocarbamate herbicides, notably EPTC (Roeth, 1986). For insecticides, research has focused on the methyl carbamates and the organophosphinothioate compound isofenphos (Felsot, 1989). Management practices involving reduced application frequency have often provided the most reliable improvements in efficacy (Felsot & Tollefson, 1990; Harvey, 1990).

The objectives of the present experiments were to determine whether the capacity of a soil for rapid carbetamide degradation declines over time and whether alternative herbicides can provide effective control. A knowledge of these factors may help to establish effective herbicide rotations.

Materials and methods

Decline of rapid carbetamide degradation

Carbetamide degradation kinetics were measured in three soils over three consecutive years (1994–96). The carbetamide application histories of the soils were: (a) previously untreated control soil; (b) one carbetamide application each year from 1989 until 12 months prior to sampling; and (c) soil from the same field as B except that the final carbetamide application was made in 1992. Soil A had a pH of 4.5 and comprised 7% silt and 10% clay. Soils B and C had a pH of 5.8 and comprised 6% silt and 8% clay. Soil samples were collected during each year at the normal time for carbetamide application (July, August) by bulking together four subsamples from each plot (10 m × 2 m, four replicates). Carbetamide (20 mg a.i. kg^{-1}) degradation was monitored using chemical (hplc-UV) and oat (*Avena sativa* L.) bioassay techniques after its application to replicate soil samples (150 g per sample), which were prepared and maintained at 15 °C as described previously (Hole *et al.*, 2001; Hole & Powles, 1997).

Most probable number (MPN) assay of carbetamide-degrading organisms

The soils sampled in 1996 for the above experiment were assayed to determine the most probable number (MPN) of carbetamide-degrading micro-organisms. The MPN assay was performed in 96 well micro-plates (500 μL $well^{-1}$) with loss of ^{14}C carbetamide (uniformly ring labelled, technical grade, Rhône-Poulenc Agrochimie, France) used to indicate degradation. Soil dilutions were prepared using soil extract broth (SEB). For SEB, 50% soil in water (wt/*v*) was mixed on an orbital shaker for 1 h, autoclaved (1 h, 120 °C) and allowed to cool. The supernatant was decanted and autoclaved (15 min, 120 °C). The initial soil dilution comprised 10 g sieved (3.9 mm) soil with 90 mL SEB mixed for 1 h on an orbital shaker (60 $rev.min^{-1}$) After allowing particulate matter to settle for 60 s, 200 μL was transferred into the first row of eight wells in the

micro-plates, which had been pre-loaded with 200 μL of SEB. Twofold serial dilutions (23) of each sample were then made. Autoclaved water (70 μL), containing carbetamide and ^{14}C-labelled carbetamide was added to each well (final concentration 70 mg a.i. L^{-1} technical grade carbetamide and 4.2×10^5 Bq L^{-1} ^{14}C carbetamide). Plates were fitted with lids, wrapped in aluminium foil and incubated at 15 °C for 28 d. A 50-μL subsample from each well was then assayed using liquid scintillation to determine remaining radioactivity. Loss of 20% or more of the applied radioactivity was indicative of carbetamide degradation, although typically 50% of the radioactivity was lost. MPN values were obtained using published tables (Rowe *et al.*, 1977) and adjusted for soil water content.

Degradation of herbicides other than carbetamide

Degradation of six commercially available herbicides was measured in the soil showing enhanced biodegradation of carbetamide (b) and the previously untreated soil (a). Soil samples (150 g) were treated (20 mg a.i. kg^{-1}) with carbetamide (technical grade, Rhône-Poulenc), or commercial formulations of propham (Clopham, 750 g a.i. kg^{-1}, WP, Agchem), chlorpropham (Allicide, 500 g a.i. L^{-1}, SC, Lane), EPTC (Eptam, 720 g a.i. L^{-1}, SC, ICI Cropcare), propyzamide (Kerb, 500 g a.i. kg^{-1}, WP, Agchem), diuron (Aguron 500, 500 g a.i. L^{-1}, SC, Agchem) or simazine (Simazine 500, 500 g a.i. L^{-1}, SC, Agchem). Soil samples were incubated at 15 °C and subsampled (25 g) at 0 and 14 d after treatment. Reversed-phase hplc was used to quantify all herbicides except propyzamide. For hplc analysis, samples were extracted as previously described for carbetamide (Hole & Powles, 1997), eluted using a water:acetonitrile gradient and monitored using UV absorbance (carbetamide, propham and chlorpropham, 233 nm; simazine and diuron, 254 nm; EPTC, 220 nm). Residual propyzamide was determined by gc-ECD (Varian Star 340cx, DB5 column, 5 μL inj., Vol., inj. 260 °C, col. 180 °C, det. 300 °C) (Adler *et al.*, 1972). Propyzamide was extracted by shaking with methanol (25 mL) for 1 h. A subsample (500 μL) of the supernatant was centrifuged (12,566 × ***g***, 5 min) to remove particulate matter prior to injection.

Field efficacy of alternative herbicides

In 1993, plots (10 m × 2 m, four replicates) infested with *Vulpia bromoides* L. (silver grass), located in the field from which soils B and C were later sampled, were treated with (post-emergence, early tillering) commercial formulations of five herbicides. Treatments were: carbetamide (2.1 kg ha^{-1}, Carbetamex, 700 g a.i. kg^{-1}, WP, Rhône-Poulenc), propham (3.75 a.i. kg ha^{-1}), propyzamide (1.0 kg a.i. ha^{-1}), diuron (1.5 a.i. kg ha^{-1}) or simazine (2.0 kg a.i. ha^{-1}); and untreated controls. The herbicides were applied using a propane pressurized hand held boom sprayer (200 L ha^{-1}, 2.5 bar). Simulated rainfall (5.6 mm) was applied 24 h after treatment. The numbers of *V. bromoides* which remained 118 d after treatment were assessed by counting plants in four randomly selected quadrats (0.25 or 0.025 m^2 – depending upon plant density) within each plot.

Results and discussion

Changes in the rate of carbetamide degradation

The results in Fig. 1 indicate that upon cessation of carbetamide application, the enhanced capacity of the soil for carbetamide biodegradation declined slowly with time. In soil where

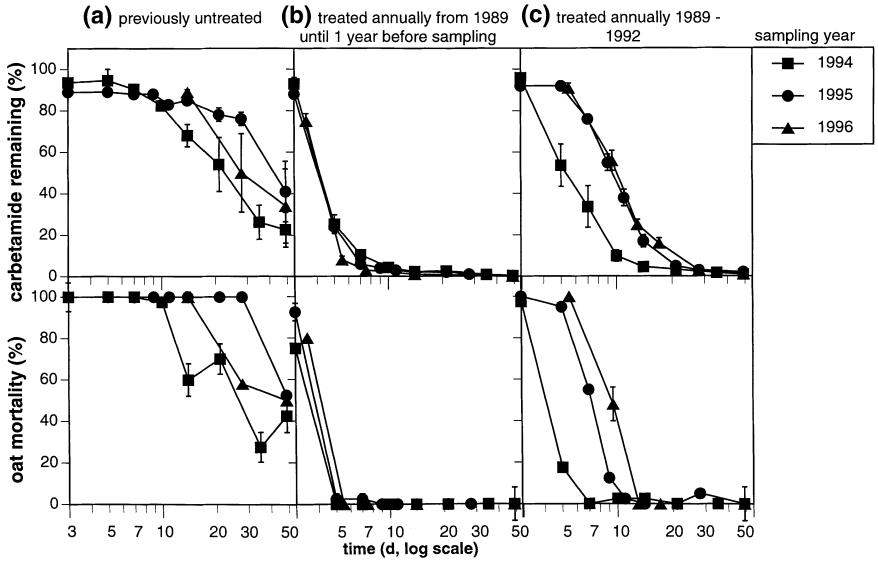

Fig. 1 Carbetamide degradation kinetics in three soils collected in 1994, 1995 and 1996. Soils were (a) previously untreated with carbetamide (b) treated once annually with carbetamide until 12 months before sampling, or (c) treated annually with carbetamide from 1989 to 1992. Degradation kinetics were determined using hplc and oat bioassay. Each point is the mean of four replicates. Vertical bars indicate ±standard error of the mean.

annual carbetamide application ceased in 1992, the rate of carbetamide degradation subsequently declined over the 3 years 1994–96 (Fig. 1c). There was an increase in DT_{50} (time taken for 50% degradation) from 5.4 d in 1994 to 10.2 d in 1996. Also, the lag time before rapid carbetamide degradation commenced increased from 3 d in 1994 to 5 d in 1995 and 1996. When annual application of the herbicide continued, the capacity for rapid carbetamide degradation persisted (Fig. 1b). In the previously untreated control soil (no carbetamide history, Fig. 1a) the carbetamide degradation rate was always slower than in soils previously treated with carbetamide (Fig. 1b and c). Using DT_{50} values derived by linear interpolation to compare degradation rates amongst soils (Table 1), the decline in the soil's capacity for rapid carbetamide degradation after cessation of carbetamide application (b) was not caused by seasonal variability. Seasonal variability would be evident in one or both of the other soils (a,c).

Table 1 Carbetamide DT_{50} in days as influenced by prior carbetamide application and time since last carbetamide application, as determined in 1994, 1995 and 1996. DT_{50} values were determined using linear interpolation between appropriate data points

Year of sampling	Previously untreated with carbetamide (no carbetamide history) (a)	Treated with carbetamide annually from 1989 until 12 months before sampling (b)	Treated with carbetamide 1989–92 (c)
1994	23.0	4.3	5.4
1995	43.6	4.2	9.6
1996	28.0	4.2	10.2

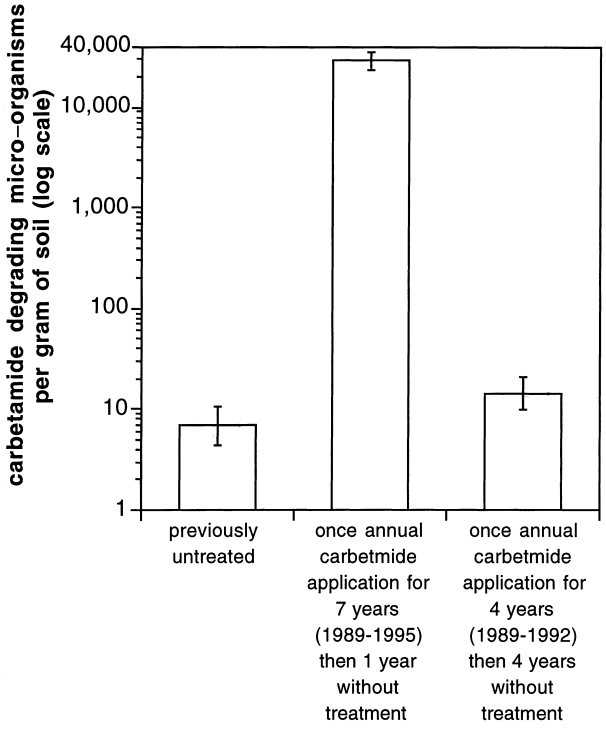

Fig. 2 Most probable number (MPN) of carbetamide-degrading micro-organisms in three soils when sampled in 1996 with differing carbetamide application histories as described for Fig. 1. Each point is the mean of four replicates. Vertical bars indicate ±standard error of the mean.

The data obtained from the oat bioassay confirmed those from the chemical analyses, with germinating oat mortality closely reflecting carbetamide concentration in the soil. When degradation rate was rapid (Fig. 1b), oat mortality was very low for seeds planted 5 d or more after carbetamide treatment, demonstrating that carbetamide levels had dropped below the lethal concentration. In contrast, for previously untreated soil (Fig. 1a), mortality was 100% for oats seeded 7–16 d after carbetamide addition, demonstrating that residual amounts remained at phytotoxic levels. When there had been a 2–4 year interval since the most recent carbetamide application, there was a slower rate of carbetamide degradation in the soil (Fig. 1c), resulting in herbicidal concentrations of carbetamide persisting for longer.

When sampled in 1996, MPN analysis of the soils provided evidence that rapid carbetamide degradation (Fig. 1) is linked to an elevated soil population of carbetamide-degrading micro-organisms (Fig. 2). Soil in which carbetamide degradation occurred rapidly (Fig. 1b) had a higher population of carbetamide-degrading micro-organisms (29 000 g^{-1}) than the untreated control soil (7 g^{-1}), in which carbetamide degraded at a much slower rate. Conversely, in soil in which the most recent carbetamide application had been 4 years prior to sampling, there were only 14 carbetamide-degrading micro-organisms g^{-1} soil as determined using MPN enumeration. Our results are similar to those obtained with other pesticides. For example, the population of 2,4-D degrading organisms was observed to decline to levels similar to those of the control soil after 4 years in a Canadian soil (Smith & Aubin, 1994). However, although the population had declined to levels similar to the control, when 2,4-D was applied it still degraded at a faster rate than when applied to the control soil. Rapid degradation was demonstrated to be caused by dramatic increases in the number of 2,4-D metabolizing organisms (Holben *et al.*, 1992).

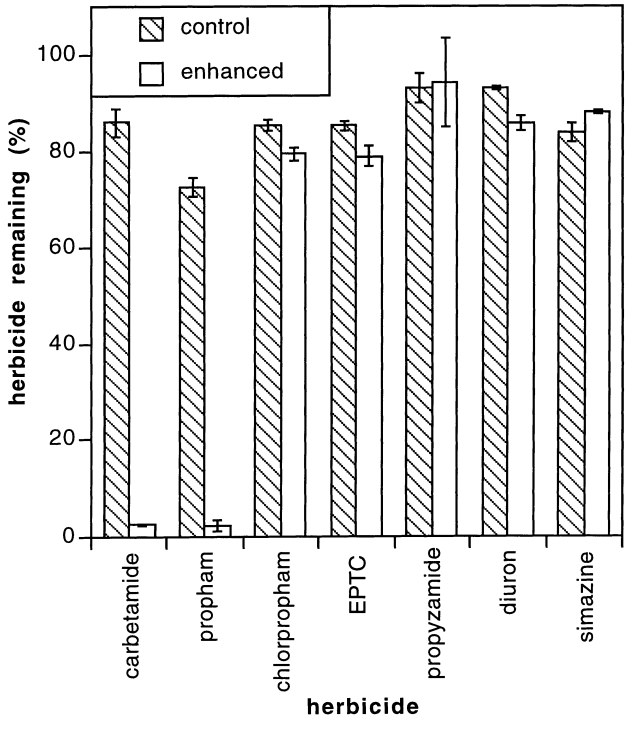

Fig. 3 Degradation of alternative herbicides in soil which degrades carbetamide at an enhanced rate. The percentage degradation after 14 d is compared to soil previously untreated with carbetamide. Each bar is the mean of four replicates. Vertical bars indicate ± standard error of the mean.

Degradation of other herbicides

The results in Fig. 3 show that propham degraded more rapidly when applied to the soil that had a carbetamide application history than when applied to the control soil. After 14 d, only 2% of the applied propham remained in the soil exhibiting rapid degradation of carbetamide, whereas about 73% remained in the control soil. In contrast, the degradation rate of the other herbicides (chlorpropham, EPTC, propyzamide, diuron and simazine) was similar in both soils (79–94% remaining 14 d after addition) and was thus independent of prior carbetamide history.

Field efficacy of alternative herbicides

Field results confirmed the failure of carbetamide to control a natural infestation of *V. bromoides* in soil with a carbetamide pre-treatment history (3400 plants m^{-2}, 118 d after treatment, Fig. 4). Carbetamide normally gives good control of this weed species (Stephenson & Madin, 1984). Whilst carbetamide treatment did reduce *V. bromoides* plant density, the level of control achieved was inadequate. Competition with the crop would probably lead to large reductions in seed yield and replenishment of the weed seedbank. Similarly, propham failed adequately to control *V. bromoides* when applied to soil with a carbetamide pre-treatment history (Fig. 3). In contrast to the results with carbetamide and propham, application of propyzamide, a herbicide with a similar mode of action and soil activity to both carbetamide and propham, gave complete control with no plants surviving in any plot 118 d after treatment. Application of either diuron or simazine also provided effective weed control.

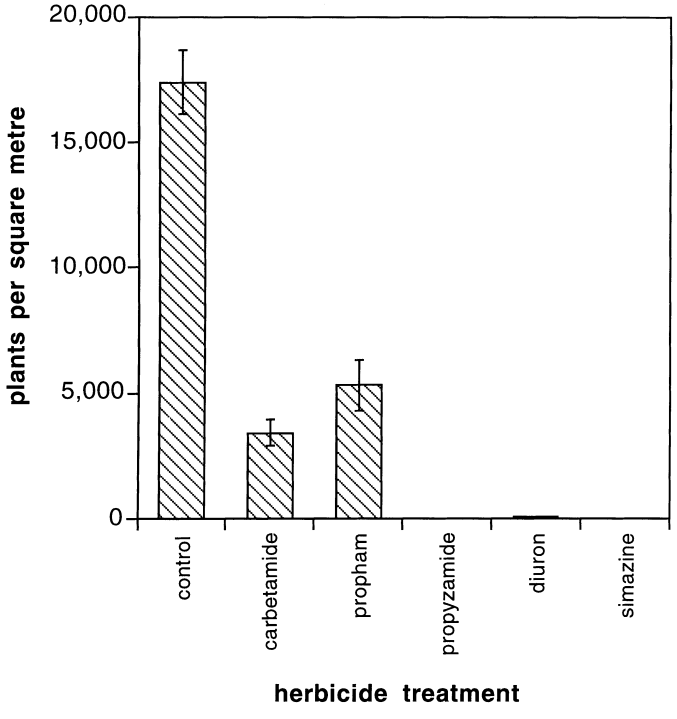

Fig. 4 Efficacy of five herbicides to control a naturally occurring infestation of *V. bromoides* in a field in which carbetamide failure occurred due to rapid carbetamide degradation. Plots were assessed 188 d after treatment. Vertical bars indicate ±standard error of the mean.

Implications for future carbetamide use

The results presented in Fig. 1 demonstrate that in a soil in which carbetamide pre-treatments have resulted in enhanced or accelerated carbetamide biodegradation, the soil partially loses the rapid-degrading capacity after cessation of carbetamide application. This loss of carbetamide-degrading ability is almost certainly caused by a significant decline in the population of carbetamide-degrading micro-organisms within the soil (Fig. 2). It is clear from our results that less frequent carbetamide application will probably result in improved carbetamide efficacy when it is applied. However, the extent of improved weed control under a range of conditions and with a range of soil types was impossible to ascertain from the studies reported here, as only a single soil was studied in detail under laboratory conditions.

Circumstantial evidence suggests that less frequent carbetamide application will result in improved carbetamide efficacy when applied. Carbetamide failure owing to enhanced biodegradation was only noticed when application frequency increased as alternative herbicides failed owing to herbicide resistance (Heap & Knight, 1982). As carbetamide was available for at least a decade prior to failures being reported and subsequently confirmed (Hole & Powles, 1997; Hole *et al.*, 2001), it is probable that less frequent carbetamide application will result in more efficacious weed control. This is in agreement with other studies which indicate that less frequent application of other pesticides amenable to enhanced biodegradation often results in longer soil persistence (Karpouzas *et al.*, 1999) and improved weed control (Harvey, 1990; Harvey, 1991).

Investigation of herbicide rotation to provide acceptable weed control demonstrated that all alternative herbicides, except propham, provided acceptable weed control. Propham provides inadequate weed control (Fig. 4) when applied to carbetamide-treated soil owing to rapid degradation of propham in these soils (Fig. 3). Based upon the similarity of the chemical structure of propham and carbetamide, it is likely that propham is an alternative carbon and energy source for carbetamide-degrading micro-organisms. Therefore, it is probable that propham application will maintain and/or elevate the population of carbetamide-degrading micro-organisms. Reflecting this, as part of a rotation programme, propham could be substituted for carbetamide, or vice versa, but these herbicides cannot be used as part of a single rotation cycle. Herbicides which are structurally different to carbetamide, such as propyzamide, EPTC, diuron and simazine, were not degraded rapidly in soil previously treated with carbetamide (Fig. 3) and, where tested, provided acceptable control in the field (Fig. 4). The success of alternating applications of carbetamide and propyzamide, in either order, has been demonstrated previously in the field (Hole & Powles, 1997). In addition, failure of repeated propyzamide applications was also reported. Based upon the crops grown in the area where carbetamide is used, all the herbicides tested are likely to form part of any rotation involving carbetamide. These herbicides can be applied to the same crops treated with carbetamide, or to crops which are grown in rotation with carbetamide-treated crops.

Whilst rapid degradation of chlorpropham in soil that rapidly degrades carbetamide was not evident in this study, it is likely that continued rotation between carbetamide and chlorpropham would not provide a long-term solution. Bacteria capable of degrading both propham and chlorpropham have been isolated from soil (Kaufman & Kearney, 1965). Bacteria are probably responsible for rapid degradation of carbetamide (Hole *et al.*, 2001) and that these bacteria are also capable of degrading propham (Fig. 3). Therefore, in our opinion, soil bacteria could probably adapt to degrade propham, carbetamide and chlorpropham. Hence, rotation amongst these herbicides is not recommended.

Conclusion

The research reported here documents that less frequent carbetamide application will probably result in more effective weed control. Alternative herbicides are available which enable effective weed control in the years when carbetamide is not applied – supporting the frequently complementary practices of herbicide/crop rotation. Further field-based work is required to demonstrate that less frequent carbetamide application provides effective weed control in the field.

Acknowledgements

This research was supported by an Australian Grains Research and Development Corporation Junior Research Fellowship and the Rural Industries Research and Development Corporation. We would like to thank Richard Bentham for help with gc analysis.

References

Adler IL, Gordon CF, Haines LD & Wargo JP (1972) Determination of residues from the herbicide N-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide by electron capture gas-liquid chromatography. *Journal of the AOAC* **55,** 802–805.

Desmoras J, Ganter P, Owen PG, Jacquet P & Laurent M (1967) Études sur le mode d'action du phénylcarbamoyloxy-2 N-éthylpropionamide, isomère D. *Weed Research* **7,** 261–271.

Felsot AS (1989) Enhanced biodegradation of insecticides in soil: implications for agroecosystems. *Annual Review of Entomology* **34,** 453–476.

Felsot AS & Tollefson JJ (1990) Evaluation of some methods for coping with enhanced biodegradation of soil insecticides. In: *Proceedings 198th National ACS Meeting Symposium, Enhanced Biodegradation of Pesticides in the Environment*, Miami Beach, Florida, USA, 192–213.

Harvey RG (1990) Systems allowing continuous use of carbamothioate herbicides despite enhanced biodegradation. In: *Proceedings 198th National ACS Meeting Symposium, Enhanced Biodegradation of Pesticides in the Environment,* Miami, Florida, USA, 214–221.

Harvey RG (1991) Effect of herbicide rotations on enhanced herbicide biodegradation and wild-proso millet (*Panicum miliaceum*) control. *Weed Science* **39,** 607–613.

Heap J & Knight R (1982) A population of ryegrass tolerant to the herbicide diclofop-methyl. *The Journal of the Australian Institute of Agricultural Science* **48,** 156–157.

Holben WE, Schroeter BM, Calabrese VGM *et al*. (1992) Gene probe analysis of soil microbial populations selected by amendment with 2,4-dichlorophenoxy acetic acid. *Applied and Environmental Microbiology* **58,** 3941–3948.

Hole SJW, McClure NC & Powles SB (2001) Rapid degradation of carbetamide upon repeated application to Australian soils. *Soil Biology and Biochemistry* **33,** 739–745.

Hole SJW & Powles SB (1997) Reduced efficacy and enhanced degradation of carbetamide after repeated application in Australia. *Weed Research* **37,** 165–170.

Karpouzas D, Walker A, Froud-Williams R & Drennan D (1999) Evidence for the enhanced biodegradation of ethoprophos and carbofuran in soils from Greece and the UK. *Pesticide Science* **55,** 301–311.

Kaufman DD & Kearney PC (1965) Microbial degradation of isopropyl-N-3-chlorophenylcarbamate and 2-chloroethyl-N-3-chlorophenylcarbamate. *Applied Microbiology* **13,** 443–446.

Racke KD & Coats JR (1990) *Enhanced biodegradation of pesticides in the environment*. *Symposium held at 198th National ACS Meeting*, September 10–15, 1989, Miami Beach, Florida, American Chemical Society, Washington DC, USA.

Robertson BK & Alexander M (1994) Growth-linked and cometabolic biodegradation: possible reason for occurrence or absence of accelerated pesticide biodegradation. *Pesticide Science* **41,** 311–318.

Roeth FW (1986) Enhanced herbicide degradation in soil with repeated application. *Reviews of Weed Science* **2,** 45–65.

Rowe R, Todd R & Waide J (1977) Microtechnique for most-probable-number Analysis. *Applied and Environmental Microbiology* **33,** 675–680.

Smith AE & Aubin AJ (1994) Loss of enhanced biodegradation of 2,4-D and MCPA in a field soil following cessation of repeated herbicide applications. *Bulletin of Environmental Contamination and Toxicology* **53,** 7–11.

Stephenson DW & Madin RW (1984) Annual grass weed control in legume-based pastures. In: *Proceedings Seventh Australian Weeds Conference,* Perth, Australia, 350.