Weed Research, 1984, Volume 24, 327–331

Measurement of phytotoxicity of commercial and unformulated soil-applied herbicides

J. C. STREIBIG *Royal Veterinary and Agricultural University, Department of Crop Husbandry and Plant Breeding, Thorvaldsensvej 40, 1871 Copenhagen V., Denmark*

Accepted 20 September 1983

Summary: Résumé: Zusammenfassung

The relative potencies of analytical-grade lenacil (99% ai) and its commercial formulation, Venzar® (80% ai), technical-grade ethofumesate (96%) and its commercial formulation Nortron® (21% ai) were evaluated using oats (*Avena sativa* L. cv. Selma) as test plant.

In all experiments the dose–response curves of the pure herbicide and its commercial product were parallel. The average potency of Venzar relative to lenacil, assayed twice in a sandy loam, was 0·80 and thus equal to the expected 0·80. The average potency of Nortron relative to ethofumesate, assayed in a sandy loam and a loamy sand, was 0·25 and close to the expected value of 0·21.

The implication of using the technique of parallel-line assay in herbicide studies is discussed and its potential use in other contexts than the present one is suggested.

Mesure de la phytotoxicité de certains herbicides d'application au sol en formulation commerciale et non-formulés

Les activités relatives du lénacile analytique (99% ma) et de sa formulation commerciale, Venzar® (80% ma), ainsi que de l'éthofumesate technique (96% ma) et de sa formulation commerciale, Nortron® (21% ma) ont été évaluées en utilisant l'avoine (*Avena sativa* L. cv. Selma) comme plante d'essai.

Dans toutes les expériences, les courbes dose–réaction de l'herbicide pur et de son produit commercial étaient parallèles. L'activité moyenne du Venzar par rapport au lénacile dans deux essais sur un limon sablonneux était 0,80, donc indentique à la valeur pronostiquée (0,80). L'activité moyenne du Nortron par rapport à l'éthofumesate dans des essais sur limon sablonneux et sable limoneux était 0,25, donc proche de la valeur pronostiquée (0,21).

La discussion porte sur le potentiel de l'emploi d'une technique d'essai en lignes parallèles dans des études herbicides et propose son emploi en d'autres circonstances que celles de cette expérience.

Messung der phytotoxischen Wirkung von formulierten und unformulierten Bodenherbiziden

Die relative Aktivität von Lenacil (99% AS) und dem Handelsprodukt Venzar® (80% AS), sowie die von Ethofumesat (96%) und dem Handelsprodukt (Nortron® (21% AS) wurden mit Hafer als Testpflanze untersucht.

In allen Versuchen verliefen die Dosis–Wirkungskurven des Wirkstoffs und des entsprechenden Handelsprodukts parallel. Die Durchschnittsaktivität von Venzar betrug in zwei Tests mit sandigem Lehm 0,80 relativ zu Lenacil und entsprach damit dem erwarteten Wert. Die Durchschnittsaktivität von Nortron betrug in Tests mit einem sandigem Lehm und einem lehmigen Sand relativ zu Ethofumesat 0,25 und stimmte mit dem Erwartungswert (0,21) brauchbar überein.

Die Anwendungsmöglichkeiten des 'parallel-line-assay' für Untersuchungen mit Herbiziden wird diskutiert.

Introduction

The objective of this study was to compare the phytotoxicity of commercially formulated soil-applied herbicides with unformulated substances.

In practical spraying situations, herbicides are formulated to keep active ingredients of low solubility dispersed in the spraying solution and to increase adhesion of spray droplets to aerial plant parts as well as to facilitate rapid penetration of the herbicide through the cuticle (Polon, 1973). Formulations may also affect availability and bioactivity of soil-applied herbicides (Schmidt & Pestemer, 1980; Horowitz, 1977) although the main effects of formulations are thought to be indirect (Walker, 1980).

One way of assessing whether formulated substances in commercial products only exert a dilution effect upon root-absorbed herbicides is to apply the principles of parallel-line assay (Finney, 1978, 1979). A parallel-line assay, previously used in context with herbicides (Sampford, 1952; Blackman, 1952; Allott & O'Neill, 1969; McKinlay *et al.*, 1972), assumes that if the plant response is plotted against log-dose of two preparations of the same herbicide, the two curves ought to be identical except for a horizontal displacement defining their relative potency. The parallel-line assay further assumes that the horizontal displacement of the formulated herbicide relative to analytical-grade material is a measure of dilution brought about by the formulants (Finney, 1979). In the simple case of linear response on log-dose a test for parallelism is easily established. Herbicide dose–response curves, however, are rarely linear, but sigmoid (Allott & O'Neill, 1969; Streibig, 1980).

In the non-linear case, Finney (1979) talks about generalized parallelism which is accomplished if the curves of a standard (e.g. analytical grade) and a test preparation (e.g. a commercial product) are identical in all regression parameters except for that causing horizontal location.

If the requirements of a parallel-line assay are met, the relative potency is independent of the response level considered and the conclusions are general over the whole experimental dose range (Sampford, 1952).

Materials and methods

Analytical-grade lenacil (99%) and Venzar (wettable powder 80% w/w lenacil) were assayed twice in a sandy loam (3·4% organic matter, 15·3% clay, 16·3% silt, 64% sand), and technical-grade ethofumesate (96%) and Nortron (emulsifiable concentrate 21% w/w) in the sandy loam and a loamy sand (2·1% organic matter, 7·4% clay, 7·6% silt, 23·9% sand), using oats as test plant. Six doses of each herbicide were used. The doses of standard lenacil (analytical grade) and ethofumesate (technical grade) were based on mg ai. kg^{-1} soil, whereas doses of the test preparations, Venzar and Nortron, were based on mg formulated material kg^{-1} soil.

Twenty-five oat seeds were sown in pots containing 500 g soil with admixed herbicides. The pots were watered with 100 ml 25% Hoagland nutrient solution and placed in growth chambers with a photoperiod of 16 h at 20°C and a 1-h transition to and from a night temperature of 10°C. A complete randomized block design with four blocks was employed; the untreated control was replicated twice within each block.

Within each experiment a logistic regression model of dry matter (U) on log-dose (x) (Finney, 1979),

$$E(U) = (D-C_i)/(1+\exp(-2(a_i+b_i x)))+C_i,\ b_i<0, \quad (1)$$

was fitted simultaneously to the data from standard preparation and test preparations of the herbicides. Equation (1) is a four-parameter logistic model and is capable of fitting over the whole feasible dose range and thus allows for assay-to-assay variability and unpredicted potencies (Vølund, 1978). D denotes the upper limit and C denotes the lower limit at zero dose and at large doses, respectively. The parameter b_i denotes the slope of the logit-transformed response curves whilst a_i determines their horizontal location. The assumption of parallel dose responses requires that the D, C, and b parameters of equation 1 are similar for the standard and test preparations. The fitting was assessed by an approximate test for lack of fit using the pure error from the analyses of variance and by graphical analyses of residuals. A more detailed description of the statistical analyses used and the method of imposing constraints on parameter are found elsewhere (Streibig, 1983a, b). There were no significant block effects in the experiments.

Results

The assumption of parallel lines neither affected the tests for lack of fit nor the graphical analyses of residuals. The lower limits of responses to lenacil and Venzar were greater than those of ethofumesate and Nortron (Table 1, Fig. 1), and fitting with or without constraints of C and b scarcely affected ED_{50} (defined as a_i/b, i.e. the dry matter production half-way between the upper and lower limits of the curves) (Table 1); the greatest divergence was about 10% for Nortron in the sandy loam. The assay-to-assay variation for lenacil and Venzar when assuming parallel lines in the sandy loam was 15 and 28%, respectively. Ethofumesate appeared to be about 15% more potent in the loamy sand than in the sandy loam whereas the variation of Nortron between soils was 40%.

The parallel regression lines (Figs 1–3) of herbicide preparations made an estimate of relative potency independent of response level. As C, D and b were similar for standard preparation (S) and test preparation (T) within the experiments the linear logit-transformed dry matter (Y) in response to log-dose (Fig. 4).

$$\begin{aligned} Y &= 0{\cdot}5 \ln[(D-U)/(U-C)], \\ Y_s &= -(a_1+bx), \\ Y_t &= -(a_2+bx), \end{aligned} \tag{2}$$

determines the relative potency (R),

$$R = \text{antilog}\ ((a_2-a_1)/b),$$

which covered the entire dose range employed.

The relative potency (R) can, however, easily be incorporated into the regression model by a reparametrization of (2). Hence:

$$\begin{aligned} Y_s &= -(a+b\cdot\log(z)), \\ Y_t &= -(a+b\cdot\log(Rz)), \end{aligned} \tag{3}$$

z being the actual dose and then R is readily obtained as the parallel displacement of the test preparation curve relative to the standard preparation curve (Table 2).

Discussion

The different lower limits of the lenacil and ethofumesate curves (Fig. 1, Table 1) were obviously caused by the different mode of action of the two herbicides. Lenacil being an inhibitor of photosynthesis (Fedtke, 1982) first affected dry-matter production when seedlings became autotrophic. Ethofumesate, on the other hand, probably commenced its phytotoxic effect as soon as it was absorbed by the seedling as evidenced by severely stunted plants already showing foliar deformations in the germination phase. Although the primary mode of action of ethofumesate is still unknown it is probably associated with inhibition of meristems (Fryer & Makepeace, 1977).

In neither soils were the relative potencies of ethofumesate and Nortron far from the expected

Table 1 Regressions of dry matter on log-dose (x)

Herbicide	D	C	logit $= -(a+bx)$	ED_{50}*	ED_{50}†
		Sandy loam			
Lenacil	0·865 (0·045)‡	0·171 (0·014)	$1{\cdot}887+2{\cdot}508x$ (0·295) (0·439)	0·177	0·169
Venzar	do	do	$1{\cdot}851+2{\cdot}508x$ (0·290)	0·183	0·188
Ethofumesate	do	(0·015) (0·003)	$0{\cdot}010+2{\cdot}279x$ (0·081) (0·177)	0·990	1·094
Nortron	do	do	$-1{\cdot}267+2{\cdot}279x$ (0·173)	3·597	3·238
Lenacil	0·860 (0·020)	0·215 (0·005)	$2{\cdot}821+3{\cdot}413x$ (0·221) (0·295)	0·149	0·146
Venzar	do	do	$2{\cdot}152+3{\cdot}413x$ (0·170)	0·234	0·233
		Loamy sand			
Ethofumesate	0·916 (0·038)	0·013 (0·003)	$-0{\cdot}142+2{\cdot}490x$ (0·083) (0·173)	1·140	1·082
Nortron	do	do	$-1{\cdot}753+2{\cdot}490x$ (0·184)	5·058	5·444

* Based on the parallel-line fit.

† Based on the model allowing different estimates of a, b, and C parameters.

‡ Standard error of parameters.

do: ditto.

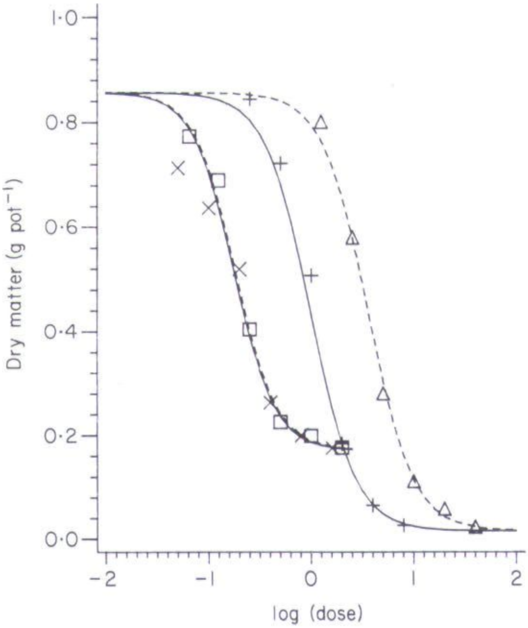

Fig. 1 Dose–response curves of dry matter on lenacil (X—X), Venzar (□- -□), ethofumesate (+—+), and Nortron (△- -△) in the sandy loam.

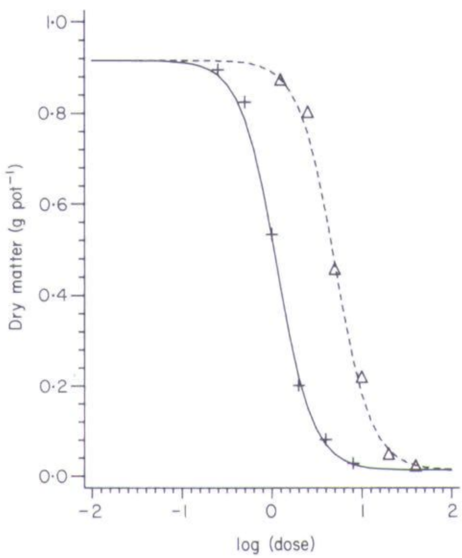

Fig. 2 Dose–response curves of dry matter on ethofumesate and Nortron in the loamy sand. Symbols as in Fig. 1.

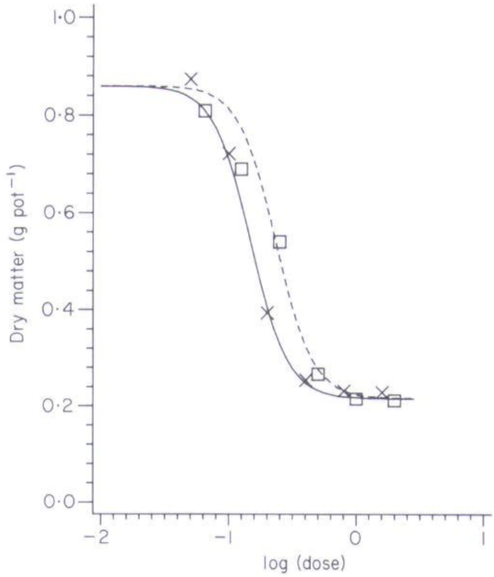

Fig. 3 Dose–response curves of dry matter on lenacil and Venzar in the sandy loam. Symbols as in Fig. 1.

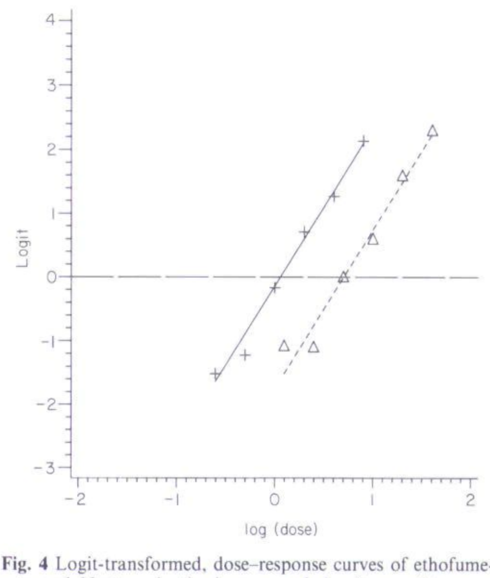

Fig. 4 Logit-transformed, dose–response curves of ethofumesate and Nortron in the loamy sand. Logits equal to zero represent ED_{50}. Symbols as in Fig. 1.

0·21. The average *R* over soils was 0·25. This also applies to the relative potency of lenacil and Venzar in one experiment in the sandy loam, whereas the estimated potency of 0·64 in the other experiment was different from the expected 0·80. The average *R* over the two experiments was, however, 0·80 (Table 2).

In conclusion there is no reason to believe that the formulations of lenacil and ethofumesate exerted any effect on the active ingredients because the proportion of active ingredients were in the same magnitude when measured by chemical methods and by bioassays. The kind of

Table 2 Estimated relative potencies of assays

Soil	Relative potency	
	Lenacil/Venzar	Ethofumesate/Nortron
Sandy loam	0·967 (0·253)*	0·275 (0·033)
	0·637 (0·079)	
Loamy sand		0·225 (0·021)

* Approximate 95% confidence interval.

bioassay presented here can of course be extended to products of unknown contents of active ingredients.

The parallel-line-assay method could easily be extended, for example, to herbicide degradation experiments to measure the rate of degradation (Allott & O'Neill, 1969). Dose–response curves with herbicide residues in which the herbicides are degraded to non-phytotoxic compounds ought to be parallel. Parallel lines may also be obtained for compounds having different modes of action. For example dose–response curves for TCA and chloridazon or for TCA and metamitron have been found to be parallel (Streibig, 1983b). The action of TCA, however, is associated with lipid metabolism whereas chloridazon and metamitron are inhibitors of the photosynthesis (Fedtke, 1982). Consequently, parallel-line assumption is a necessary but not a sufficient condition for assuming similar action of the assayed herbicides.

Slopes of the logit-transformed, dose–response curves, however, can only be compared when the upper and lower limits are similar. If data are available from different sources such as different soil types or laboratories, conversion to percent of the untreated control, for example, may overcome the difficulties of comparing different curves.

Acknowledgments

I wish to express my thanks to Du Pont de Nemours Nordiska AB and Fisons-Schering A/S, for providing the herbicides and the Danish Agricultural and Veterinary Research Council for partial financial support.

(Received 11 April 1983)

References

Allott D.J. & O'Neill J.A. (1969) A parallel line assay method for the determination of herbicide soil residues. *Record of Agricultural Research*, **18,** 21–28.

Blackman G.E. (1952) Studies in the principles of phytotoxicity. I. The assessment of relative potency. *Journal of Experimental Botany*, **3,** 1–27.

Fedtke C. (1982) *Biochemistry and Physiology of Herbicide Action*. Springer-Verlag, Berlin.

Finney D.J. (1978) *Statistical Method in Biological Assay*. C. Griffin, London.

Finney D.J. (1979) Bioassay and the practice of statistical inference. *International Statistical Review*, **47,** 1–12.

Fryer J.D. & Makepeace R.J. (1977) *Weed Control Handbook, Vol. 1.* Blackwell Scientific Publications, Oxford.

Horowitz M. (1977) Specific phytotoxicity of surfactants. *Proceeding EWRS Symposium Methods of Weed Control and their Integration*, 79–86.

McKinlay K.S., Brandt S.A., Morse P. & Ashford R. (1972) Droplet size and phytotoxicity of herbicides. *Weed Science*, **20,** 450–452.

Polon J.A. (1973) Formulation of pesticidal dusts, wettable powders and granules. In: *Pesticide Formulations* (ed. W. Van Valkenburg), pp. 143–234. Marcel Dekker, New York.

Sampford M.R. (1952) Studies in the principles of phytotoxicity. II. Experimental designs and techniques of statistical analysis for the assessment of toxicity. *Journal of Experimental Botany*, **3,** 28–46.

Schmidt R.R. & Pestemer W. (1980) Plant availability and uptake of herbicides from the soil. In: *Interactions between Herbicides and the Soil* (ed. R.J. Hance), pp. 179–201. Academic Press, London.

Streibig J.C. (1980) Models for curve-fitting herbicide dose response data. *Acta Agriculturae Scandinavica*, **30,** 59–64.

Streibig J.C. (1983a) Joint action of root-absorbed mixtures of DPX-4189 and linuron in *Sinapis alba* L. and barley. *Weed Research*, **23,** 3–9.

Streibig J.C. (1983b) Fitting equations to herbicide bioassays: using the methods of parallel line assay for measuring joint action of herbicide mixtures. *Symposium Bioassays in Weed Science, Berichte aus dem Fachgebiet Herbologie der Universität Hohenheim*, **24,** 183–193.

Walker A. (1980) Activity and selectivity in the field. In: *Interactions between Herbicides and the Soil* (ed. R.J. Hance), pp. 203–222. Academic Press, London.

Vølund A. (1978) Application of the four-parameter logistic model to bioassay: comparison with slope ratio and parallel line models. *Biometrics*, **34,** 357–365.