Rate of decline of chlorfluazuron concentration in the fat of cattle

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Objective To determine the rate of decline of chlorfluazuron (CFZ) concentration in the fat of cattle.

Design A field depletion study.

Animals Fifteen steers that had become contaminated with CFZ through eating cotton trash or cotton leaf pellets derived from CFZ-treated cotton crops.

Procedure Fat samples were collected from the cattle at about 3 week intervals according to a schedule where each animal was sampled on four occasions up to 340 days after removal from the contaminated feed source.

Results When the effects of dilution are removed CFZ concentrations were found to decline slowly for about 200 days. Depletion was minimal between 200 and 340 days.

Conclusion According to this trial, CFZ-contaminated, non-lactating cattle which have finished growing will remain contaminated. Field experience has not supported this conclusion.
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Key words: Chlorfluazuron, cattle, rate of decline.

CFZ	Chlorfluazuron
MRL	Maximum residue limit
GPC	Gel permeation chromatography
GC/MS	Gas chromatography/mass spectrometry
HPLC	High pressure liquid chromatography

CFZ (Helix 40 ULV ICI Crop Care, 1 Nicholson Street, Melbourne, Victoria) is a chitin inhibitor first registered in Australia in 1989 for use in controlling *Helicoverpa* species (cotton bollworm and native budworm) in cotton crops. CFZ residues were first detected in cattle slaughtered at a New South Wales abattoir in October 1994 (G Williamson personal communication). Subsequent investigations found residues in cattle from both New South Wales and Queensland (D Byrne personal communication). Cattle were exposed to CFZ when they were fed cotton trash or cotton leaf pellets from CFZ-treated cotton crops mostly as a drought feed, or when they grazed cotton stubble. Some pastures adjoining cotton crops or crop-dusting air strips also became contaminated through spray drift.

CFZ has a high logarithmic octanol-water partition coefficient (log p^{ow} 5.8),[1] which is considered highly lipophilic.[2] In Australia the MRL for CFZ has been set at 1 mg/kg in the fat of cattle meat and at 0.1 mg/kg for edible cattle offal. Australia's trading partners, in particular Japan, Korea, Canada and the USA, have not set tolerances for CFZ in animal products. Consequently concentrations of CFZ greater than 0.2 mg/kg in Australian export meat products are considered unacceptable (RR Biddle personal communication).

When the CFZ residues were first found, cattle producers who owned CFZ-contaminated cattle sought advice on CFZ excretion rates in cattle. There are no published reports on CFZ depletion in any species. This trial is designed to determine the rate of depletion of CFZ concentration in the fat of cattle.

Materials and methods

Fifteen cattle were selected from farms that had fed cotton trash or cotton leaf pellets to their cattle. The cattle, selected on the basis of their CFZ residue concentration, were all British breed (Hereford, Angus or Murray Grey) steers about 2 years of age.

The cattle were run together in a paddock of about 18 ha. Because of drought conditions they were also fed a restricted grain-based ration throughout the trial of about 7.5 kg per head per day.

The cattle were assigned to one of five groups so that each group contained one steer with a low CFZ fat concentration, one with a medium and one with a high concentration within the range of residue concentrations studied.

A sample of fat was collected from each of the cattle. They were weighed and had fat depth measured on the first day of the trial, which was about 30 days after the cattle had been removed from contaminated feed. The 30 day delay was used because New South Wales field experience with CFZ-contaminated cattle had suggested that CFZ residues can increase for a period, possibly as long as 60 days, after the cattle are removed from the source of contamination (D Byrne personal communication). Each group of animals had a fat biopsy taken on two further occasions and had fat samples collected at slaughter according to the schedule given in Table 1. This schedule allowed the rate of decline of the different initial residue concentrations to be monitored throughout the study. The interval between each biopsy or slaughter date was about 3 weeks.

Fat biopsies were taken using the method of Saville et al[3]

Table 1. Days on which fat samples were collected after the removal of the steers from CFZ contamination.

Group	Days			
	Sample 1	Sample 2	Sample 3	Slaughter
1	30	48	152	256
2	30	68	173	278
3	30	89	194	299
4	30	111	216	320
5	30	133	35	340

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except that 2% lignocaine was used as anaesthetic instead of dry ice. Body weight was measured using an electronic scale and fat depth measured using an EZI SCAN back fat meter.[4]

Fat samples were analysed for CFZ concentration by the method described by Armishaw et al[5] except that an amino column clean-up was used instead of the GPC clean-up and a GC/MS was used instead of a HPLC. The detection limit for this method is 0.01 mg/kg.

The residue and body weight data were analysed to determine the shape of the mean depletion curve and the rate of depletion. Depletion was modelled after correcting the observed residue concentration for dilution to give a residue concentration that might be expected if there had been no increase in body fat.

The correction for dilution was based on the allometric equation which estimates fat from body weight by the formula:

$$\text{total fat} = A \times \text{body weight}^{1.69}$$

where A is a constant for the animal.[6] The corrected concentration at time t is given by

$$CFZ_t = OCFZ_t \times (lw_t / lw_1)^{1.69},$$

$OCFZ_t$ being the observed residue concentration, CFZ_t the adjusted residue concentration, lw_t the body weight at t and lw_1 the initial body weight.

The statistical requirement is to combine the short segments (four points) from each animal's profile of CFZ concentration over time into a continuous mean curve. To do this, individual animal effects are represented in the model to account for variance amongst animals and the correlations among residuals. The random coefficients strategy of Palmer et al[7] was used to represent excretion of CFZ. Diagnostic plots of the data indicated that concentrations did not approach zero and simple first-order kinetics were not applicable. The three-parameter Mitscherlich function was a suitable function for describing depletion but estimates of parameters are unstable unless it is reparameterised to a version where the estimates have good statistical properties. Ratkowsky[8] recommends a model of the form

$$CFZ_{it} = a_i + (b_i - a_i)(1 - k_i^{(m-1)}) / (1 - k_i^{(n-1)})$$

with subscripts denoting animal i and sampling time t.

The symbol n is the sample size (four in this case), a and b are the expected values of the curve at chosen points t_1 and t_2, (0 and 260 days) and $m - 1 = (n - 1)(t - t_1) / (t_2 - t_1)$.

The mean curve is

$$CFZ_t = \alpha + (\beta - \alpha)(1 - \kappa^{(m-1)}) / (1 - \kappa^{(n-1)})$$

where κ, α and β are weighted means of the κ_i, a_i, b_i respectively. The details of calculating the weights are given in Palmer et al.[8]

The initial concentration is estimated by $\hat{\alpha}$, the concentration at 260 days is estimated by $\hat{\beta}$ and the rate of depletion by $\hat{\kappa}$. For given initial value (α) and rate parameter (κ), the depletion curve can be constructed by estimating β using formulae for the conditional expectation and variance of β, given α and κ, see Anderson.[9]

Results

On day 1 of the trial the cattle had a mean body weight of 447 kg (range 380 to 510 kg) and a mean fat depth of 8 mm (range 4 to 13 mm). The three animals remaining on the final slaughter date (day 310) weighed 628, 700 and 714 kg and had fat depths of 12, 24 and 16 mm respectively.

When the trial ended, 340 days after the animals had been removed from the source of contamination, only those animals with initial small CFZ residues (about 1 mg/kg) had a decreased concentration of 0.2 mg/kg, which is acceptable for export meat products.

For all animals, the estimate of minimum concentration was significantly greater than zero, implying that CFZ persists in the animal.

The observed CFZ concentration for each animal is shown in Figure 1.

The estimates of κ, α and β for the mean curve are 0.43 ± 0.20, 1.66 ± 0.09 and 0.77 ± 0.12 respectively. The covariances amongst the estimates (necessary for the predictions) are $\text{cov}(\kappa,\alpha) = -0.007$, $\text{cov}(\kappa,\beta) = -0.013$ and $\text{cov}(\alpha,\beta) = 0.002$.

The formulae for expected values at t_2= 260 β and their variances, given initial values (α) and $\hat{\kappa} = 0.0428$ are :

$$\beta_0 = E(\beta/\alpha, \hat{\kappa}) = 0.77 + 0.048 \times (\alpha_0 - 1.66)$$

$$se(\beta\backslash\alpha, \hat{\kappa}) = 0.10$$

In Figures 2 and 3, combined excretion and dilution curves are drawn for animals whose initial CFZ concentrations were 0.9 and 2.1 mg/kg respectively. The dilution factors were derived from their body weights using the allometry equation. The dilution factor is $(lw_{t-1}/lw_t)^{1.69}$.

Discussion

As CFZ is highly lipophilic we assumed that it was present only in the fat of the cattle. It would be excreted in milk because it is bound to the milk fat. Consequently lactating cows can have declining CFZ concentrations but their calves will absorb that CFZ, creating a further problem.

Concentration can be diluted independently of depletion. However, halving the concentration requires a 50% increase in body fat. Figures 2 and 3 imply that depletion is minimal after 200 days and that total depletion does not occur. The animal with initial concentration of 0.9 mg/kg had a CFZ concentration of 0.37 mg/kg at the end of the trial. The depletion model

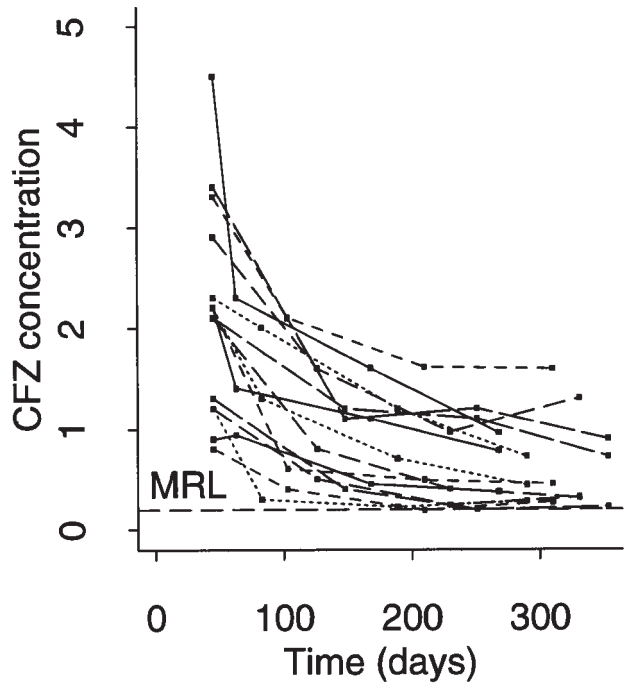

Figure 1. CFZ concentration in the fat of 15 steers during the trial.

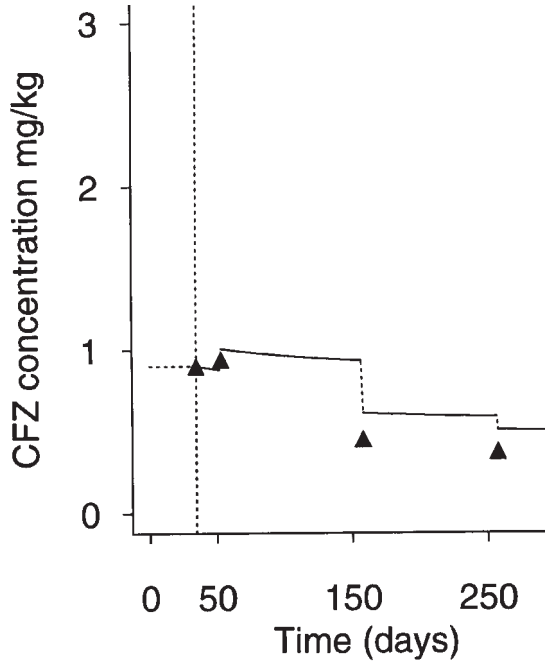

Figure 2. Combined effects of depletion and dilution for a steer with initial CFZ concentration of 0.9 mg/kg. Depletion is represented as the smooth curves and dilution by the vertical adjustments at the end of each sampling interval. The concentration is plotted as triangles.

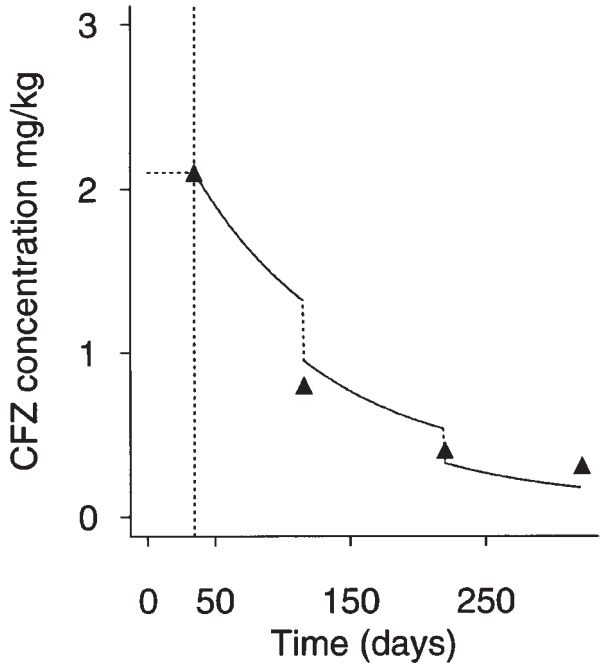

Figure 3. Combined effects of depletion and dilution for a steer with initial CFZ concentration of 2.1 mg/kg. Depletion is represented as the smooth curves and dilution by the vertical adjustments at the end of each sampling interval. The concentration is plotted as triangles.

predicted a final concentration of 0.73 mg/kg and dilution accounts for the further reduction in concentration. The other animal had a predicted depletion of 2.1 to 0.79 mg/kg and its body weight increase (402 to 622 kgs) further reduced concentration to 0.31 mg/kg via dilution.

Thus where there is potential for dilution, the combination of depletion and dilution may achieve the Australian MRL of 1 mg/kg in 250 days for cattle whose initial concentration did not exceed 2.2 mg/kg. For higher initial values, predictions from these data are unreliable; the sample included some cattle in which the concentration did reach 1 mg/kg and some cattle in which it did not.

A mathematical model is desirable for summarising the data and predicting beyond the sample. Data such as these are highly variable, and the model is limited in its ability to predict the depletion from initial CFZ concentratioms greater than about 2.5 mg/kg. Where such prediction are required, larger sample sizes will be needed.

Monitoring of a wide range of CFZ-contaminated cattle has revealed that by October 1997 few, if any, cattle in New South Wales had residues over 0.2 mg/kg (D Byrne personal communication).

This study highlighted the importance of differentiating the contribution of depletion and dilution when predicting reductions in residue concentrations.

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