IMIDOCARB DIPROPIONATE

First draft prepared by

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ADDENDUM

To the Imidocarb Dipropionate residue monograph prepared by the 50th meeting of the Committee and published in FAO Food and Nutrition Paper 41/11, Rome 1999

The Committee in its review at the 50th Meeting requested the following information for evaluation in 2001:

- Depletion studies in non-lactating and lactating cattle using the recommended dose of imidocarb dipropionate for subcutaneous injection, with analysis of samples using the proposed regulatory method which includes the enzymatic digestion step, for comparison with the findings of the radiolabel study with respect to marker residue.
- A depletion study in sheep, using the recommended dose and mode of administration of imidocarb, for identification of marker residue and target tissues.

In response, the sponsor has provided additional information on an analytical method for the determination of imidocarb residues in bovine edible tissues and milk and applied the method in studies of the residue depletion of imidocarb in milk and edible tissues of cattle treated with the injectable product. The sponsor did not provide any additional residue data for sheep.

Residue Depletion Studies (with unlabelled drug)

Cattle

A depletion study was conducted under GLP in which 18 crossbred beef cattle (9 female, 9 male) each received a single subcutaneous injection of imidocarb dipropionate at the recommended therapeutic dose of 3.0 mg/kg bw (Nolan-Smith, 2001). The treated animals ranged in weight from 202 to 290 kg on the day prior to treatment and from 242 – 397 kg at slaughter. The controls, housed separately, weighed 200 – 272 kg on the day prior to treatment and 217 - 383 kg at slaughter. From the treated animals, eight of each sex were selected and 2 of each sex were killed at 30, 60, 90 and 180 days post-dosing. A pair of untreated control animals (1 female, 1 male) was killed with each of the 30-day and 180-day groups of treated animals in the depletion trial. Liver, kidneys, injection site and samples of muscle and fat were collected from each animal at slaughter and analyzed by a liquid chromatographic procedure which measures extractable residues after digestion with protease. Details of the method are given in a subsequent section of this report. Analytical results are reported as imidocarb free base, corrected for analytical recovery as determined from the intra-day assay data during validation. Recoveries were also calculated for each batch of samples analyzed, based on fortified blank tissues included with each batch.

Table 1. Imidocarb free base residues in tissues of cattle which received a single injection (SC) of imidocarb at 3.0 mg/kg bodyweight.[1]

Withdrawal time (days)	Mean recovery corrected imidocarb concentration (mg/kg)				
	Liver	Kidney	Muscle	Fat	Injection Site[2]
30	4.10 ± 0.87	13.94 ± 4.48	0.64 ± 0.09	0.09 ± 0.02	0.56 ± 0.25
60	1.36 ± 0.24	4.58 ± 1.35	0.27 ± 0.01	0.03 ± 0.01	0.28 ± 0.10
90	1.48 ± 0.62	3.71 ± 1.06	0.22 ± 0.04	0.02 ± 0.01	0.32 ± 0.06
180	0.39 ± 0.06[3]	0.92 ± 0.34	0.09 ± 0.04[4]	0.02 ± 0.01[5]	0.09 ± 0.04

[1] Performance characteristics of the analytical method for all edible tissues are given in Table 2.

[2] The mass of each injection site was adjusted to 300 g by multiplying the actual mass of the injection site by the recovery corrected concentration of imidocarb determined and dividing by 300.

[3] Mean of three results which were below the LOQ of 0.70 mg/kg; no residues were detected in the fourth sample, with an LOD of 0.20 mg/kg.

[4] Three of 4 results averaged were below the LOQ of 0.10 mg/kg, but above the LOD of 0.004 mg/kg; the fourth sample contained residues above the LOQ.

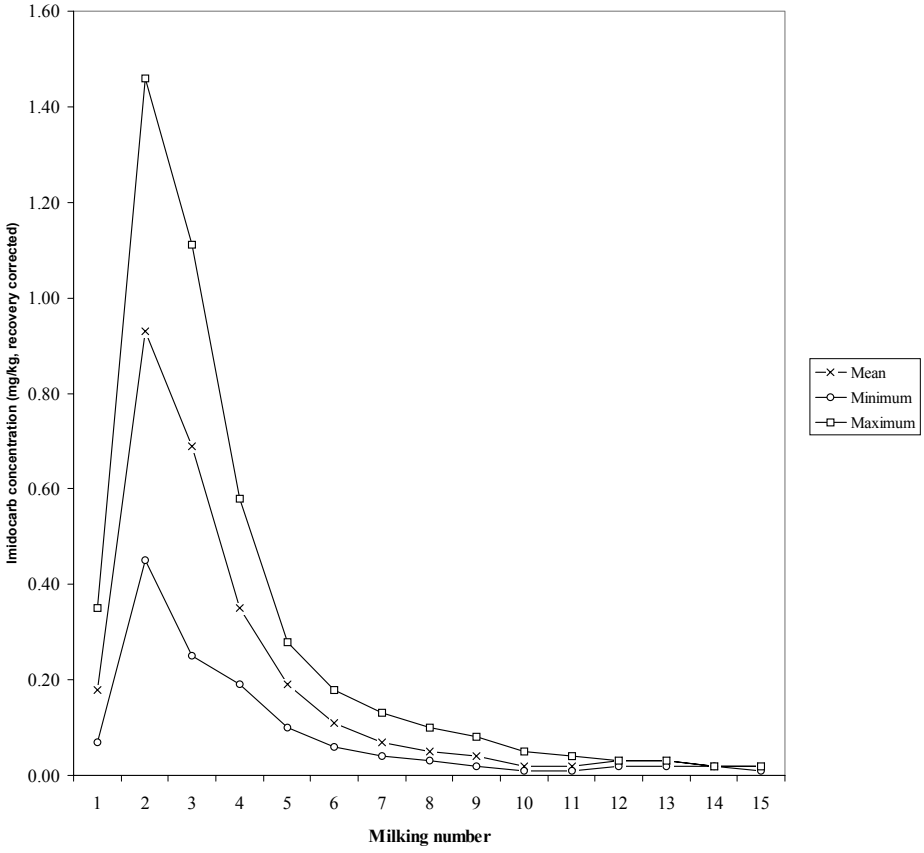
[5] Mean of three results, one of which was below the LOQ of 0.02 mg/kg; no residues were detected in the fourth sample, with an LOD of 0.006 mg/kg.

The results (Table 1) demonstrate that treatment with protease to release bound residues reveals a different residue distribution pattern in tissues, consistent with the results seen in the GLP study using ^{14}C-imidocarb dipropionate reviewed

by the 50th Meeting of the Committee. These results are therefore considered to more accurately reflect the results which would be determined in applying the proposed regulatory method to field samples and indicate that kidney is the preferred target tissue.

Lactating dairy cattle

Figure 1. Depletion of imidocarb free base residues in milk from cows which received a single SC injection of 3.0 mg/kg bw imidocarb dipropionate



In a GLP study, twenty-five lactating dairy cattle (504 – 750 kg at treatment) each received a single subcutaneous injection of imidocarb dipropionate at the recommended dose of 3.0 mg/kg bodyweight at approximately 5 hours after the morning milking (Nolan-Smith & Heal, 2001). The cows were categorized as low yielding (15 liters/day, or less – 8 cows), medium yielding (15 to 20 liters/day – 9 cows) or high yielding (20 or more liters/day – 8 cows). Two milkings were sampled prior to treatment (afternoon milking, Day "-1" and morning, Day "0"). The drug was administered after the morning milking on Day "0", then samples were collected at the afternoon milking on Day "0", at each of the two daily milkings (morning and afternoon, approximately 12-hour intervals) for 27 days, and at the morning milking on Day "28", for a total of 56 milkings following drug administration. Analyses were done using a liquid chromatographic method, described in the next section of this report. Samples were initially tested from one animal from each group for days 0 – 7 to provide a probable range for analysis of the other samples. Based on these results, only samples from days 0 – 5 were analyzed for the remaining animals (with one exception, where a day 6 sample was included as residues remained near the temporary MRL of 0.05 mg/kg in the day 5- PM sample). All results were reported as corrected for recovery, using the recovery of 88% determined during method validation.. As shown in Figure 1, all milk samples contained residues <0.05 mg/kg in the afternoon milking on Day 5. Analysis of samples of milk from the representative animals selected for the initial testing showed a continuing trend to concentrations near or below the limit of quantitation of 0.02 mg/kg by the Day 7 afternoon milking.

Methods of Analysis for Residues in Tissues and Milk

The method considered by the 50th Meeting of the Committee has been modified in a GLP study to remove some interference problems encountered with fat samples and to improve precision (Croucher & Dunn, 2001). Performance data were provided for the analysis of beef muscle, liver, kidney, fat and milk. Polypropylene tubes and silanized glassware are used throughout the procedure. After addition of dimethyl imidocarb internal standard, a 5-gram test portion of tissue (or 10 g of milk) is weighed into a 50 mL polypropylene tube. Recovery controls are also fortified at this point. 1M tri-(hydroxymethyl)methylamine buffer solution is added, then test portions are macerated and incubated with protease (from bovine pancreas). The incubated test portions are cooled to room temperature, acidified with hydrochoric acid and centrifuged. The decanted extract is made basic by addition of sodium hydroxide, then partitioned with hexane/isoamyl alcohol (3:2, v/v).

After removal of the hexane by evaporation, methanol and pH7-buffer are added and the extracts are cleaned up on a weak cation exchange (carboxylic acid) solid phase extraction cartridge. The collected fraction containing imidocarb and internal standard is reduced to dryness under a nitrogen stream and the final residue is dissolved in 1M hydrochloric acid. Liquid chromatographic analysis is conducted using a C18 reversed phase column with detection of imidocarb and the dimethyl imidocarb internal standard at 260 nm. The concentration of imidocarb is determined relative to the internal standard

response, using a calibration line based on the peak area of imidocarb relative to the internal standard over a range which includes the expected concentrations to be found in the samples. A linear response is expected.

The major numerical performance characteristics determined for the various matrices are summarized in Table 2. In addition, the method was demonstrated to have the required specificity in that imidocarb could be distinguished from other substances present in control samples and from a selection of commonly used veterinary drugs which might be present as incurred residues in field samples. Standard solutions of imidocarb and the internal standard, dimethyl imidocarb, prepared in 1M hydrochloric acid, were demonstrated to be stable in refrigerated storage (approximately 4°) over 40 days. Sample extracts were stable for 9 days under the same storage conditions, while milk and tissue samples stored frozen for 1 month at -20 ^{o}C showed no loss in residue concentrations. Additional data were provided to demonstrate that the method continues to meet required performance criteria at concentrations exceeding 4x the temporary MRLs recommended by the 50th Meeting of the Committee.

The limit of quantitation (LOQ) was established for each tissue based on a fitness for purpose approach, considering both the requirement that a regulatory method should be capable of quantitatively measuring residues at concentrations one-half the MRL and also the actual concentrations of residues found in tissues in the recent depletion studies. The LOQ was defined as the lowest concentration at which acceptable accuracy and precision could be demonstrated. In the case of the LOQs reported for imidocarb residues liver and kidney, there is a much larger difference from the limit of detection (LOD) than is usually reported.

Table 2. Performance characteristics of the liquid chromatographic assay for imidocarb residues in beef tissues and milk

Performance Characteristic	Liver	Kidney	Muscle	Fat	Milk
LOD (mg/kg)[1]	0.199	0.005	0.004	0.006	0.001
LOQ (mg/kg) [2]	0.702	0.526	0.105	0.018	0.018
Recovery (%)	87 (80-94)	86 (81-90)	84 (72-94)	86 (76-96)	88 (82-96)
Precision (%)	8.1	7.9	14.0	15.9	10.6

[1] Based on analysis of 20 controls, the mean of the measured content plus 3 standard deviations
[2] The lowest concentrations tested at which acceptable accuracy (recovery) and precision were obtained

MAXIMUM RESIDUE LIMITS

In recommending MRL's, the Committee may take into account the following factors:

- An ADI of 0-10 μg/kg bw was established by the Committee at its 50th meeting, which results in an ADI of 0-600 μg for a 60-kg person.
- The percentages of marker residue to total residues determined in the study with radiolabelled compound considered by the Committee at its 50th meeting were as follows: liver, 68%; kidney, 88%; muscle, 88%; milk, 77%. As no data were available for fat, a factor based on the lowest ratio reported (in liver) was applied by the Committee at its 50th meeting. The new data considered by the Committee at its present meeting confirm the concentrations predicted from the data for total residues in fat (reviewed by the Committee at its fiftieth meeting) using this factor. The present Committee rounded the percentages and assigned factors for correction of market-to-total residues as follows: liver, 0.7; kidney, 0.9; muscle, 0.9; fat, 0.7; milk, 0.8.
- The recommended MRLs are based on data resulting from the treatment of cattle with the recommended therapeutic dose of 3.0 mg/kg bw administered as a single subcutaneous injection.
- Imidocarb free base is the appropriate marker residue, as determined by the Committee at its fiftieth meeting.
- The new data on residue depletion indicate that kidney and muscle are the recommended target tissues.
- A suitable analytical method is available for analysis of imidocarb free base residues in edible tissues of cattle and cows' milk.

Based on the above considerations, the following permanent MRLs were recommended by the Committee for edible tissues of cattle, expressed as imidocarb free base:

Muscle	300 μg/kg	Fat	50 μg/kg
Liver	1500 μg/kg	Milk (cows')	50 μg/kg
Kidney	2000 μg/kg		

The MRL's recommended above would result in a daily maximum intake of 523 μg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat and 1.5L of milk, as shown in Table 3.

Table 3. Theoretical Maximum Daily Intake of Imidocarb residues from beef and milk.

Tissue	Recommended MRL (µg/kg)	Food Factor (g)	MR/TR	Consumption (µg)
Muscle	300	300	0.9	100
Liver	1500	100	0.7	214
Kidney	2000	50	0.9	111
Fat	50	50	0.7	4
Milk	50 (µg/L)	1500	0.8	94
Total				523

APPRAISAL

The data provided by the sponsor address the requests from the 50th Meeting of the Committee for additional data on the depletion of residues in lactating and non-lactating cattle which have been treated with imidocarb dipropionate at the recommended dose and for analysis of the samples using the method with enzymatic digestion to release bound residues. Additional data were also provided to support modifications to the method reviewed by the 50th Meeting of the Committee.

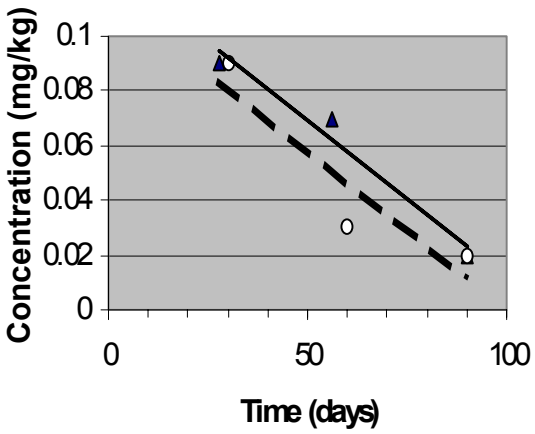
The depletion study in non-lactating cattle confirmed the general pattern of distribution of residues in various tissues shown in the earlier non-GLP studies reviewed by the 50th Meeting of the Committee. While differences in ages and weights of animals, treatment regimens, sampling times and analytical methodology make direct comparisons difficult, the data consistently demonstrate that the highest residues are found in kidney, followed by liver, muscle and fat. Liver was identified as the target tissue by the 50th Meeting of the Committee, based on results of earlier depletion studies which did not use the current procedures to release bound residues. However, the current GLP study clearly demonstrates that kidney is the preferred target tissue.

Residues are persistent. Most samples from any of the edible tissues contain detectable residues at 180 days after treatment at concentrations well below the temporary MRLs recommended by the 50th Meeting of the Committee, but all kidney samples and one liver sample at tested at 90 days post-treatment contained residues above these concentrations. The residues in kidney determined using the analysis with enzymatic digestion are higher than those reported previously, when the Committee established a higher temporary MRL for liver than for kidney. Based on the new data, the Committee considered it appropriate to adjust the MRLs to reflect the distribution of parent compound in these tissues, as reflected in the new GLP study in cattle.

As all residues in liver were below the LOQ of 0.70 mg/kg at day 180, the MRL for liver could be set at approximately 2x the LOQ, or 1.50 mg/kg. Similarly, the MRL for kidney could be adjusted to address the higher residue findings in the new study. Based on the data at Day 180, a concentration of 1.6 mg/kg could be suggested (mean + 2x std. dev.), or a "rounded up" value of 2.0 mg/kg could be proposed. This would, in effect, exchange the previous temporary MRLs for liver and kidney to reflect the actual distribution and would result a small reduction in the TMDI, as shown in Table 3.

The new data for analysis of residues in fat support the use of data from liver by the 50th Meeting of the Committee to identify a factor for correction of marker to total residues in fat. The analytical method for marker residue applied in the GLP study using ^{14}C-imidocarb propionate which that Committee used to establish factors was not able to detect marker residues in fat. Improvements to the method for application in the non-label study considered by the present Committee, however, revealed residues in fat consistent with the concentrations which would be expected if the factors were applied to the results from the earlier radiolabel study were calculated as marker residue only. The concentrations of total residue reported in the GLP study using ^{14}C-imidocarb propionate reviewed by the 50th Meeting of the Committee (Ferguson, 1996) were multiplied by the factor 0.68 to obtain predicted concentrations of the parent (marker residue). These were then plotted in comparison with the results reported in the depletion study provided for review by the present Committee (Nolan-Smith, 2001). The animals were administered equivalent amounts of the labelled and unlabelled drugs in the two respective studies, but there were small variations in the sampling times. As shown in Figure 2, the results reported in the new study with unlabelled drug agree with the predicted concentrations of parent compound calculated from the total residues reported in the earlier study. At 90 days, the predicted concentration from the radiolabel study matches the actual value determined in the new study with unlabelled drug. This substantiates the use of 0.68 as an appropriate factor for conversion of marker residue to total residue in the TMDI calculation.

Figure 2. Comparison of predicted marker residue concentration in fat calculated from radiolabel study (Ferguson, 1996) with marker residue concentrations measured in current study (Nolan-Smith, 2001)



The results of the depletion study in lactating dairy cattle follow a similar pattern to results in the earlier studies reviewed by the 50th Meeting of the Committee. The residues peak the day following treatment, then decline rapidly over the next 48 hours, after which there is a continued slow elimination at concentrations which are below the temporary MRL from Day 5 onwards in milk from cattle to which the drug has been administered. The data support a permanent MRL, with a note to national authorities that discard is advised for the next 8 milkings post-treatment. The data provided for treatment of dairy cattle at the therapeutic dose of 3.0 mg/kg bodyweight indicate that a minimum discard period of 96 hours (normally 8 milkings) is required for commingled milk from the herd, assuming one-third of the animals have been treated. Typically, therapeutic treatment would not include all animals in a herd. However, in situations where treatment of all animals in a herd with the therapeutic dose is required, a discard period of a minimum of 7 days appears prudent. The Committee considered, but did not recommend, that the discard period could be reduced in the normal circumstances of treatment of selected animals in a herd by 12 hours, or 1 milking, if the MRL for milk was established at 100 μg/kg. This would increase the TMDI to 617 μg, resulting in a theoretical daily consumption, which exceeds the ADI of 600 μg by 3%.

The sponsor has provided a suitably validated method to support the MRL for the marker residue, imidocarb free base. As noted in the previous discussion of the analytical method, the reported LODs for imidocarb residues in liver and kidney are considerably lower than the LOQs. In the case of these tissues, the reported LOQ could be better described as the "lowest calibrated level", or LCL. Laboratories wishing to apply the method to determine lower concentrations of imidocarb in liver or kidney may be able to validate the method performance at lower LOQs for these tissues. Typically, the LOQ is 2 – 3 times greater than the LOD, while in the reported validation, the LOQ for imidocarb residues is approximately 3.5 times the LOD for liver and 100 times the LOD for kidney.

The critical control points in the method, such as silylation of glassware and maintaining the SPE cartridges wet, have been identified. The recovery and precision meet the requirements for regulatory methods as established in Codex Alimentarius, Volume 3. Information on method specificity and analyte stability are provided. The method includes an internal standard, which corrects for recovery and validation data are provided to indicate that the method performs in a satisfactory manner at the temporary MRLs established at the 50th Meeting of the Committee, or at the proposed change in the recommended permanent MRLs considered by the present Committee. The method is suitable for use in a routine residue control laboratory.

REFERENCES

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