SUMMARY

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STUDY TITLE

2,4-Dichlorophenol: Relevance for 2,4-D Treated Corn Containing the DAS AAD-1 Trait

DATA REQUIREMENTS

none

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STUDY COMPLETED ON

01-NOV-2010

PERFORMING LABORATORY

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LABORATORY STUDY ID

101759

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This report provides an assessment of 2,4-dichlorophenol (DCP) as a metabolite from AAD-1 corn treated with 2,4-D herbicide. The report includes: a) the regulatory history for DCP relative to the residue definition; b) a review of the DCP toxicological database; c) an overview of 2,4-D and DCP residues from new 2,4-D MOR studies with AAD-1 corn; and d) a comparison of the amounts of DCP from AAD-1 corn compared to existing 2,4-D animal burdens for qualitative dietary assessment.

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: 2,4-dichlorophenol

Title: 2,4-Dichlorophenol: Relevance for 2,4-D Treated Corn Containing the DAS AAD-1 Trait

- STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS:

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Company: Dow AgroSciences LLC

Company Agent: D. Fonseca

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Signature: 

Date: 28 October 2010

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STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

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ABSTRACT

This report provides an assessment of 2,4-dichlorophenol (DCP) as a metabolite from AAD-1 corn treated with 2,4-D herbicide. The report includes: a) the regulatory history for DCP relative to the residue definition; b) a review of the DCP toxicological database; c) an overview of 2,4-D and DCP residues from new 2,4-D MOR studies with AAD-1 corn; and d) a comparison of the amounts of DCP from AAD-1 corn compared to existing 2,4-D animal burdens for qualitative dietary assessment.

The report concludes that existing corn and corn feed tolerances for 2,4-D are adequate to cover the use of 2,4-D on AAD-1 corn. The review of the 2,4-DCP toxicology database concludes that 2,4-DCP is no more toxic than the parent 2,4-D and therefore existing regulatory endpoints for 2,4-D are adequate for risk assessment for both 2,4-D and DCP. International trade is best served by preserving the residue definition for tolerances as 2,4-D acid only. The amount of DCP in corn grain is negligible and will have no impact on human dietary assessment. The EPA's existing RED for 2,4-D contains a slightly refined acute dietary assessment and the moderately refined dietary assessment; qualitatively there is adequate room within this risk cup to accommodate the relevant DCP residues from AAD-1 corn feeds which are <5% or less of the EPA Maximum Reasonably Balance Diet (MRBD) animal burdens for 2,4-D.

INTRODUCTION

This report fulfills a request from the Health Effects Division (HED) staff of EPA (1) for Dow AgroSciences to provide an overview of information on 2,4-Dichlorophenol (DCP) in conjunction with the Dow Herbicide Tolerant trait (DHT) project for 2,4-D uses on AAD-1 corn. DCP is an animal and plant metabolite of the herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D). AAD-1 modified corn contains the AryloxyAlkanoate Dioxygenase trait which provides rapid metabolic detoxification mediated by an α-ketoglutarate-dependent dioxygenase and thereby provides protection to certain phenoxy auxins such as 2,4-D. The herbicidal tolerance of AAD-1 corn to 2,4-D relies on increased plant metabolism through a pathway involving DCP. In December 2009 (2), EPA and Dow AgroSciences agreed on the outlined elements of this paper to be covered as: a) historical residue definition on DCP, b) toxicity summary, c) DCP within NOR and MOR studies of AAD1 corn and d) relative impact of DCP in animal diets.

The herbicide 2,4-D **(**CASRN: 94-75-7) is an aryloxyalkanic acid used globally to control broadleaf weeds in a variety of crops and on non-cropland. The mode of action for 2,4-D is to affect plant growth regulation in a manner similar to 3-indoleacetic acid. DCP (CASRN: 120-83-2) is a primary degradate of 2,4-D resulting from cleavage of the side chain leaving the resultant phenol. DCP has been observed in the metabolic and degradative pathways of: photolysis, soil degradation, anaerobic aquatic soil metabolism, and plant and ruminant metabolism (3, 4, 5), often as a minor component. DCP is also a common manufacturing intermediate of several pesticides, disinfectants and preservatives and moth-proofing products. A comprehensive review of phenols in general was compiled by ATSDR of the CDC (6) in 1999.

HISTORICAL BACKGROUND

DCP as an Animal Metabolite

2,4-dichlorophenol is an animal metabolite of 2,4-D. A summary of the levels of DCP observed in a few key animal studies are reproduced in Table 1 to provide context for the rest of this report. In EPA's December 3, 2003 MARC summary (4), the ruminant lactating goat ^{14}C 2,4-D metabolism study (7) was conducted for 3 consecutive days at 483 ppm in the diet, with results showing DCP as a minor metabolite in several tissues. Per the sheep feeding study (8) reviewed in the RED, sheep were dosed with 2000 ppm 2,4-D for 28 days, followed by residue analysis; DCP was observed in the liver and kidney above 0.05 ppm (additionally residues of both 2,4-D and DCP were below 0.05 ppm after a 6 –day withdrawal period.) Per the cattle feeding studies with beef calves (9) discussed in the RED, calves were fed 2,4-D for 28 days at 300, 1000 and 2000 ppm in the diet; DCP was observed in minor amounts. Literature from USDA in sheep and cattle (10) indicates ~ 5 fold increase of residues of DCP from liver and kidney tissues under alkaline extraction conditions. Residues of DCP in milk after dairy cows were fed 1000 ppm of 2,4-D for two weeks (11) were reported as <0.05 ppm.

Residue Definition for 2,4-D with and without DCP

Historically, U.S. EPA tolerances for 2,4-D have consisted of 2,4-D residues in plant, processed food/feed and fish as well as 2,4-D and/or DCP residues in animal commodities (meat, milk, poultry and eggs). In 1993, the EPA's Health Effects Division Metabolism Committee recommended changing the tolerances to 2,4-D *per se* for all commodities in order to harmonize with Codex maximum residue levels (MRLs) based on toxicological evaluations produced by the *WHO/FAO Joint Meeting on Pesticide Residues JMPR*. However, final procedures to change the listing in 40 CFR 180.142(a)(8) and remove DCP from the US tolerance of meat and milk were made only after several years of discussion. The official proposal to remove DCP was posted in the 2007 Federal Register (Ref 12, page 31226). This 2007 action relies on recommendations in the 2005 2,4-D RED (5) which stemmed from the 2004 HED Metabolism Assessment Review

Committee decision upholding a previous 2003 recommendation to delete the DCP from the livestock tolerance expression for 2,4-D (4,13). The MARC committee stated *2,4-DCP is not of concern for either the tolerance expression or for risk assessment at the levels expected in livestock tissues and considering the likely lower toxicity of 2,4-DCP compared to 2,4-D* (4).

Per the Residue Review (14) conducted in preparation for the 2,4-D RED:

> *The reregistration requirements for plant and livestock metabolism are fulfilled. Adequate metabolism studies are available depicting the qualitative nature of the residues in three dissimilar crops (lemon, potato, and wheat), the goat, and the hen. Based on the available data, on 9/3/03, the MARC determined that the residue of concern in plants and livestock for both tolerance expression and risk assessment purposes is 2,4-D, free and conjugated, determined as the acid (W. Hazel and L. Taylor, 12/3/03, D293128, TXR No. 0052264).*

Congruent with the 2005 RED (5), the current eCFR entry for the residue definition of 2,4-D tolerances in food under 180.142 is (15): *Tolerances are established for residues of the herbicide, plant regulator, and fungicide 2,4-D (2,4-dichlorophenoxyacetic acid), both free and conjugated, determined as the acid.* The US EPA action for removal of DCP from the US tolerance expression for livestock tissues allows for harmonization of US tolerances with international MRLs established by CODEX and other trading partners such as Japan and Europe who never included DCP in any tolerance expression.

Current International MRLs for 2,4-D

The United States is the top exporter of corn (or maize) to the world based on a two-year average of trade value statistics from the United Nations comtrade database (16). Continued international harmonization of the 2,4-D MRL values as well the residue definition for enforcement is critical for the US grower. **The residue definition for 2,4-D is currently harmonized around the world** to be 2,4-D acid or 2,4-D plus other forms (esters or salts) expressed as acid **and does not include DCP** (17). Per Appendix A—International MRLs for 2,4-D in Corn, **Global MRLs for 2,4-D on corn are reasonably harmonized around the world with a majority set at 0.05 ppm** and a range of 0.01 to 0.5 ppm. US congruency with Japan and CODEX MRLs is especially

important; based on an "HS-1005 Maize (corn)" search in the UN comtrade database (16), Japan is the top single largest country importer of US corn. Collectively, countries which rely on CODEX MRL values for trade comprise the largest market sector for US exported corn).

TOXICITY SUMMARY FOR DCP

A thorough evaluation of the DCP toxicology database has been conducted. This review relies primarily on the public IUCLID dataset (18) which is the single largest body of pertinent DCP information. Additional information was obtained through a search of the open literature.

Acute Studies

DCP has relatively low acute toxicity by oral (540-4000 mg/kg bw; cat III), dermal (780 mg/kg bw; cat II), and inhalation (0.97 mg/l; cat III) routes of exposure. Concentrated materials are generally corrosive eye and skin irritants. No studies assessing dermal sensitization for DCP have been reported.

Subchronic Studies

The IUCLID was reviewed for repeat dose studies and the most relevant studies for repeat dose toxicity with DCP were three subchronic studies. In the first study, mice were administered DCP *via* drinking water for three months that corresponded to 0, 50, 143 or 491 mg/kg bw/day in females and 0, 40, 114 or 383 mg/kg bw/day in males. No adverse effects were reported at any dose. Two additional subchronic studies in rats and mice were conducted by NTP (19). Rats and mice were fed DCP in the diet for three months at doses of 2500, 5000, 10,000, 20,000 or 40,000 ppm. In the mouse NTP subchronic study, lethality occurred at the highest dose in mice within three weeks and mild liver damage was observed at all remaining doses, manifested as necrosis and multi-nucleated hepatocytes. The LOAEL for mice was 2500 ppm (750 mg/kg

bw/day). In the rat NTP study, bone marrow atrophy/degeneration was observed near the limit dose in female rats at 10,000 ppm (800 mg/kg bw/day) and male rats at 20,000 ppm (1500 mg/kg bw/day). The NOAEL for rats was 5000 ppm (400 mg/kg bw/day).

Genotoxicity

A series of *in vitro* and *in vivo* genotoxicity studies were conducted for DCP. Negative *in vitro* results were reported in the Ames assay and for DNA damage and repair, HGPRT, unscheduled DNA synthesis and chromosomal aberration endpoints. Both *in vivo* studies were negative, which included a mouse micronucleus and sister chromatid exchange assays. Conflicting *in vitro* results were reported for chromosomal aberration, a mammalian gene mutation assay, a mouse lymphoma assay, and a sister chromatid exchange assay. The data for these assays would not be considered acceptable by today's OPPTS and OECD standards. The positive mouse lymphoma assay was reported by NTP (19), but upon review the "small" increases seen at an acceptable level of toxicity were not significant and did not meet the trigger criteria for a positive response, whereas the "large" increase in mutant frequency was only observed at excessive cytotoxicity (unacceptable per today's standards). The CHO SCE reported by NTP (19) reported equivocal results. Most importantly, the more relevant *in vivo* sister chromatid exchange assay was negative. The positive mammalian cell gene mutation assay in Chinese hamster cells (20) was not referenced properly in IUCLID and was instead a novel assay for spindle disturbances and not for gene mutations, which is not a validated assay. **Overall, the weight of evidence supports that DCP is not genotoxic.**

Carcinogenicity

The carcinogenic potential of DCP was evaluated in rats and mice in a NTP study (19). Fischer 344 rats were exposed to 5000 or 10,000 ppm DCP in males and 2500 or 5000 ppm in females. B6C3F1 mice were exposed to 5000 or 10,000 ppm DCP. Mean body weights for high-dose rats were generally 5-11% lower than controls, and mean body weights of high-dose female rats were

6-12% lower than controls. Mean body weights of high-dose male mice were generally 3-9% lower than controls, and mean body weights of high-dose female mice were considerably more than 10% lower than those of controls throughout the study. No compound-related increases in the incidence of neoplastic lesions were observed in either study (19).

Reproductive and Developmental Toxicity

Reproductive and developmental toxicity with DCP has also been evaluated. In a two-generation reproductive toxicity study, rats received diets containing 0, 500, 2000, or 8000 ppm 2,4-dichlorophenol (21). A NOEL for systemic maternal toxicity of 500 ppm (33.4 mg/kg) was reported, based upon decreases in feed consumption and body weights in the 2000 ppm group. A NOAEL for reproductive toxicity of 2000 ppm (134 mg/kg) DCP was established based on a decrease in number of implants and litter sizes in the F1 parental/F2 offspring in the presence of significant adult toxicity at 8000 ppm (543 mg/kg).

Aoyama *et al*. (21) reported a treatment-related increase in uterine weights, but these findings are not considered treatment-related for the following reasons: 1) the degree of increase of uterine weights was very small, 2) adult uterine weight data is complicated by the fact that there is a considerable range of physiological state in the females at the time of necropsy, due to variability in time since actively nursing pups and the fact that the animals are not synchronized in their estrous cycle, and 3) weight of evidence from several reproductive assays (including the uterotrophic assay, which is specifically designed to detect estrogenic/anti-estrogenic effects on the uterus) showed no effect on the uterus. An additional discrepancy with the author's interpretation of the data is the "slight acceleration in female sexual maturation" which was a mean acceleration of vaginal opening by 0.7 days; this was not statistically identified and of a sufficiently small magnitude so as to be considered not biologically significant.

The lack of any treatment-related effect of DCP on the above parameters, estrous cyclicity, mating index, fertility index, gestation index and length, primordial ovarian follicle counts, sperm parameters, anogenital distance or hormone measurements as well as only a small

decrease in the number of implants and resultant litter size in the presence of significant maternal toxicity collectively demonstrate that DCP does not elicit observable endocrine activity at the dose levels tested. Evidence from several reproductive assays (ER binding, uterotrophic, human ER genes + estrogen responsive element and others) demonstrating a lack of estrogenicity (18) further support this conclusion. Although two assays reported estrogenic effects (Yeast two-hybrid assay, Human MCF-7 cells), these were the exception and demonstrated effects were either at very high doses or in phenotypically-altered cells (breast tumor cells) (18).

In a developmental toxicity study, Rodwell *et al.* administered DCP to female F344 rats at doses of 0, 200, 375 or 750 mg/kg/day on gestation days 6-15; these animals were cesarean-sectioned on day 20 (22). DCP was not considered to be teratogenic; while there was a reported delay in fetal development, this was secondary to maternal toxicity at 750 mg/kg/day. The maternal LOAEL was 200 mg/kg bw/day, and the developmental NOAEL was 750 mg/kg bw/day.

Conclusion

In conclusion, based on the review of the DCP toxicology database, DCP is no more toxic than the parent, 2,4-D (Table 2) based on the 2005 EPA RED (5).

DCP INFORMATION FROM NOR AND MOR STUDIES OF AAD1 CORN

Nature of Residue (NOR) Studies

For AAD-1 corn, a Nature of Residue (NOR) study was conducted with radiolabeled 2,4-D applied as the 2,4-D dimethylammonium (DMA) salt to evaluate the plant metabolic pathway for 2,4-D treatment (23). [^{14}C]-2,4-D was applied to plots of AAD-1 maize (Event 278) at 1X maximum seasonal rate of 3.36 kg a.e./ha *via* three applications (each 1.12 kg a.e./ha at pre-emergence, V4 and V8 growth stages). The maize was grown outdoors to maturity and plot

maintenance simulated typical cultural practices. Identifiable residues were associated with the 2,4-D acid herbicide and the phenol metabolite DCP as either free moieties or conjugates. The low levels of free DCP relative to the conjugated forms indicate conjugation is rapid and a preferential route of metabolism. Extensive metabolism of 2,4-D was demonstrated by incorporation or encapsulation of radioactivity into natural plant constituents such as starch, pectin, and lignin. The proposed metabolic pathway for AAD-1 corn is found in Figure 1. Importantly, no new or novel metabolites were identified and these results are confirmatory of a probe NOR study of 2,4-D in AAD-1 corn (a non-priority transgenic event 474) (24) and congruent with the literature documentation that in plants the overall metabolic routes involve (3): *cleavage of the side chain, extension of the side chain, hydroxylation of the aromatic ring and formation of conjugates with carbohydrates, amino acids and peptides*. A discussion of the key details for individual matrices within the recent AAD-1 NOR study follows.

Grain and Cobs

Mature grain and cobs were collected 18 September 2009, with a pre-harvest interval (PHI) of 66 days after the third application. At maturity, the grain and cobs contained a Total Radioactive Residue (TRR) of 0.032 µg a.e./g and 0.014 µg a.e./g, respectively with a LOQ of 0.013 ppm. This value is 100-fold less than the TRR observed in the immature forage or mature fodder and indicates very little translocation of residue into the grain or cobs. Starch was isolated from a separate portion of grain and ~21% of the TRR in grain (0.007 mg a.e./kg) was associated with starch. Due to the low level of extractable radioactivity in grain, no further characterization of the observed residue was possible. However, it is noted that this TRR value is less than the current US tolerance for 2,4-D acid in corn grain at 0.05 ppm (15). Additionally, similar results were found in the 2008 NOR study: a) overall activity in the grain was very low, b) the 2,4-D acid was the only identifiable residue (the DCP was not detectable) in the grain, but at a level less than 10% of the TRR and c) a large portion of the grain TRR was associated with the starch fraction (24).

Animal Feeds: Forage and Fodder

Forage was collected on 25 August 2009 with a PHI of 42 days after the third application. Mature fodder was collected alongside the grain and cobs with a PHI of 66 days. Forage contained a TRR of 3.020 µg acid equivalents/g (LOQ of 0.013 ug a.e./g). The mature fodder TRR was 4.179 µg a.e./g. TRR values on the order of 10% were associated with bound residues in the forage and fodder; these were subjected to bound residue determinations including pectin, acid-detergent fiber, lignin, and cellulose isolation. The major radioactive component identified in forage and fodder was the 2,4-D acid which comprised, 77.4% and 56.5% of the TRR values, respectively. Glucose and disaccharide conjugates of DCP identified in forage and fodder summed to ~20% TTR; a very small percentage (~2.0% of TRR) of free DCP was observed in these animal feeds. The observed residues are less than the current US tolerance for 2,4-D acid in corn forage and stover at 6 and 50 ppm, respectively (15).

Sample	2,4-D		DCP		DCP-conjugate	
	% TRR	a.e. mg/kg	% TRR	a.e. mg/kg	% TRR	a.e. mg/kg
Forage	77.4%	2.338	2.3%	0.069	20.0%	0.603
Fodder	56.5%	2.362	1.7%	0.072	20.2%	0.844

Magnitude of Residue (MOR) Studies

For AAD-1 field corn, a Magnitude of Residue study was conducted in field corn in 2009 across 25 sites in the US and Canada (25, 26). Grain, processed products, plus stover and forage animal feeds were collected. For completeness, the analyses for the MOR trials were designed to include observed residues of both 2,4-D and DCP. 2,4-DCP is not part of the residue definition for 2,4-D, but it is of interest because the herbicidal tolerance of AAD-1 corn to 2,4-D relies on increased plant metabolism through a pathway involving 2,4-DCP. Method performance was demonstrated, with concurrent recoveries ranging from 77-109% for 2,4-D, 61-112% for DCP. The MOR study contains analysis for quizalofop analytes as well, but those results are not relevant for this paper.

2,4-D Observed Residues

Results reported in Table 7 of Ref (26) have been summarized in Table 3 and confirm that for 2,4-D use in AAD-1 corn, the resulting 2,4-D residues are covered by the existing US tolerances for 2,4-D in grain, processed products and animal feeds. There were no detectable residues of 2,4-D in processed products of oil, meal, flour, starch and grits. Aspirated grain sample at the 2X treatment range had a detectable residue at 0.021 ppm in one of two aspirated grain trials; the observed value is far below the current EPA tolerance of 40 ppm.

For grain, only one quantifiable residue was noted in one of the two replicates at one of the 25 sites; the replicate sample for this same trial site was non-detectable; therefore the average 2,4-D residue for that one site is 0.034 ppm. Three other detectable but non-quantifiable observations were noted, the remainder and majority of samples had non-detectable residues of 2,4-D in the grain. The associated stover sample residues for this trial (site 8) were significantly lower than this grain value. Contamination of this sample seems likely. **The outlier nature** of this one grain residue value is clearly **observable in Figure 2**. Dow AgroSciences believes it is **not appropriate to include this single outlier value for the 2,4-D corn tolerance** consideration, especially given that the replicate was ND, that the HAFT value is compliant with the existing grain tolerance and corn grain is a blended commodity (27). For completeness, Table 3 contains an overview of the residues with and without this outlier sample.

Animal feed results were reviewed in the NAFTA MRL calculator for forage and stover residues of 2,4-D. The mean+3SD method results in a 8 ppm tolerance for stover at 90 day PHI and a 3 ppm value for forage at 40 day PHI. Variability in the data set for the 60 day forage samples resulted in a 4 ppm calculated value. Per Table 3 all values are within current tolerances already established for 2,4-D (15).

DCP Observed Residues

Per Table 7 of Ref 26, the 2009 AAD-1 corn MOR study also included analysis for the 2,4-D phenol DCP plant metabolite. These results are also summarized in Table 3. In grain, the majority of samples analyzed had no detectable levels of DCP, and no grain samples at the 1X

application rate had quantifiable residues of DCP; in treatment 4 (the 2X application rate), there was one quantifiable residue just above the LOQ at 0.0138 ppm. In the stover and forage animal feeds, observed DCP residues ranged from non-detectable to a maximum of 5.8 ppm in stover. The lack of relevant impact of this additional DCP in animal feeds will be explored in the following section and demonstrated to be ~ 5% or less of the animal Maximum Reasonably Balanced Diet (MRBD) animal burdens for 2,4-D.

RELATIVE IMPACT OF DCP IN ANIMAL AND HUMAN DIETS

In this section the difference between the historical 2,4-D maximum theoretical dietary burdens (MTDB) and newer Maximum Reasonably Balanced Diet (MRBD) calculations is first explored and then the MRBD values are used to place the AAD-1 corn DCP residues values in context.

2,4-D Maximum Reasonably Balanced Diet (MRBD)

Current tolerances in meat and milk products for 2,4-D (15) are based on projections of animal dietary burdens during the RED process (5). At that time, the maximum theoretical dietary burdens (MTDB) for beef and dairy cattle were calculated at 874 ppm, and the MTDB for poultry and swine were listed as 1.6 ppm (14). Revised calculations of animal dietary burden have been used to project the animal burden for cattle, swine and poultry of 2,4-D based on the newer procedures for Maximum Reasonably Balanced Diet (MRBD) animal burden for US EPA (28). The diets for each model animal are presented separately in Tables 4 and 5. Based on current procedures, these new cattle burdens (Table 4) are revised downward significantly from the RED and therefore contain 25% additional room for any new exposures, before current meat/milk tolerances would need to be increased. For poultry and swine tolerances, there is a small amount of room for new exposures before tolerances need reconsideration as well (Table 5).

Lack of 2,4-D Corn Contributions to Animal Burden of MRBD

In Table 6, the potential contribution to the MRBD animal burden based on the current 2,4-D tolerances in corn are calculated. It is clear that the potential corn feed contributions are lower than those of other existing animal feeds and thus corn does not contribute to the MRBD values in Tables 4 and 5. For example, in the CC category of complex carbohydrate feeds, potential burden from corn grain at 0.05 ppm is significantly lower than the contribution of other grains with a tolerance of 2 ppm such as barley, millet or wheat. Likewise in the R or roughage category of feeds, the high values for 2,4-D tolerances in the crop group 17 (grass forages and hay at 360 and 300 ppm, respectively), result in much higher potential contributions to the cattle burdens than corn feeds in the roughage category. As a consequence of this review, no revisions are needed to the tolerances for animal meat, milk and egg commodities for use of 2,4-D in AAD-1 corn.

2,4-D DCP Residues vs 2,4-D Maximum Reasonably Balanced Diets

These 2,4-D MRBD animal burdens can also be used to assess the additional DCP exposure from AAD-1 corn in context of potential exposure. Consideration of DCP contributions in animal feeds relative to 2,4-D are explored here in the context of dietary risk assessment at the suggestion of EPA (2). In Table 7, the potential DCP contribution to the MRBD animal burden is calculated. In the context of dietary risk assessment, the use of the DCP HAFT is assumed appropriate based on general recommendations from the DEEM manual reflecting EPA policies (29) and based on the fact the animal feeds are classified as partially blended commodities (27). Comparison of the predicted DCP dietary exposures in Table 7 to those in Table 6, indicates the 2,4-D tolerances are an adequate estimate of combined 2,4-D and DCP and residues in animal commodities for any dietary risk analysis.

Importantly it is noted in Table 8 that the percentages of **additional animal exposure from DCP AAD-1 corn exposure relative to the existing 2,4-D animal burdens are a small (1 to <5% in cattle) to negligible component (<0.01% in poultry and swine) of animal burden for**

2,4-D used to set tolerances. Based on the toxicology review, for a risk assessment these contributions could be assessed against the existing 2,4-D reference doses, because DCP is found to be no more toxic than the parent. Qualitatively the approximate 25% extra room between the former RED animal burden and the revised MRBD burden is more than adequate to accommodate the additional <5% DCP exposure in animal feeds.

Relative Impact of AAD-1 Corn DCP in Human Diets

From Table 3, there is negligible new DCP exposure *via* grain for human consumption. (Note In the existing RED assessment (30), the primary corn commodities which contributes to the estimated exposure is corn syrup; this is a conservative representation of exposure given observable residues in corn syrup are negligible based on the 1998 and 1999 analyses from USDA PDP program (31) in which 454 samples were analyzed across 2 years for 82 pesticides and no observable residues were observed in any of the samples.) Moreover, there is a <5% impact of DCP on the 2,4-D meat and milk tolerances for human consumption. The 2,4-D RED indicates that the existing "acute dietary assessment was only slightly refined" and that the "chronic dietary assessment was moderately refined"(5); these statements imply that there is room to accommodate any slight changes in risk assessment needed to address the DCP from AAD-1 corn, if the EPA desires. EPA Ref (30) states that EPA could use additional percent crop treated data to further refine the existing assessment. Qualitatively, no or a minimal revision to existing dietary assessment for 2,4-D would be needed to accommodate the small additional amount of DCP in animal feeds.

CONCLUSIONS

The report concludes that existing corn and corn feed tolerances for 2,4-D are adequate to cover use of 2,4-D on AAD-1 corn. International trade is best served by preserving the residue definition for tolerances as 2,4-D acid only. The review of the DCP toxicology database clearly indicates that DCP is no more toxic than the parent, 2,4-D and therefore if EPA pursues a risk

assessment of DCP from AAD-1 corn, it is assumed that existing regulatory endpoints for 2,4-D are adequate for risk assessment for both 2,4-D and DCP. The additional amount of DCP in corn grain is negligible and will have no impact on human dietary assessment. EPA's slightly refined acute dietary assessment and the moderately refined dietary assessment have adequate room to accommodate the relevant DCP residues from AAD-1 corn feeds which are <5% or less of the MRBD animal burdens for 2,4-D. Qualitatively, no or a minimal revision to existing dietary assessment for 2,4-D would be needed to accommodate the small additional amount of DCP in animal feeds.

REFERENCES

1. Meeting between Dow AgroSciences and EPA, August 24, 2006.

2. Meeting between Dow AgroSciences and EPA, December 15, 2009.

3. T. Roberts, ed., Metabolic Pathways of AgroChemicals, Part One: Herbicides and Plant Growth Regulators, The Royal Society of Chemistry, Cambridge, 1988.

4. US EPA, 2,4-D HED MARC Decision Document, DP Barcodes D293119 and D293128, Chemical ID No 030001, Case no, 0073, Meeting Date 9/3/03, Memorandum TXR No. 0052264, December 3, 2003.

5. US EPA, Reregistration Eligibility Decision for 2,4-D, EPA738-R-05-002, June 2005.

6. ATSDR of CDC, Agency for Toxic Substances and Disease Registry, Toxicological Profile for chlorophenols, http://www.atsdr.cdc.gov/toxprofiles/tp107.html , accessed January 14, 2009.

7. M. Guo and S. Stewart, Metabolism of Uniformly 14C-Ring Labeled 2,4-Dichlorophenoxyacetic Acid in Lactating Goats, **MRID 42749701**, Dow Chemical Report, GH-C 3032, April 22, 1993.

8. D. J. Jensen and P. W. Miller, Residues of 2,4-D Dichlorophenoxyacetic acid and2,4-Dichlorophenol in tissue of sheep fed 2,4-D, **MRID 00068893**, Dow Chemical Report, GH-C 460, July 27, 1971.

9. P. W. Miller, D. J. Jensen, and W. M. Gentry, Residues of 2,4-Dichlorophenoxyacetic Acid and 2,4-Dichlorophenol in Tissues of Beef Calves Fed 2,4-D (Final Report), **MRID 00068892**, Dow Chemical Report, GH-C 457, June 1, 1971.

10. D. E. Clark, J. S. Palmer, R. D. Radeleff, J. R. Crookshank, F. M. Farr, Residues of Chlorophenoxy Acid Herbicides and Their Phenolic Metabolites in Tissues of Sheep and Cattle, J. Agric. Food Chem, **23** (3), 1975, http://pubs.acs.org/doi/abs/10.1021/jf60199a032, accessed Sept 30, 2010.

11. P. W. Miller, Residues of 2,4-D and 2,4-Dichlotphenol in milk form Cows Fed 2,4-D in conjunction with Dowco 290, **MRID 00059034**, Dow Chemical Report, GH-C 804, April 9, 1975.

12. US Federal Register, Vol 72, No. 108, 31221, June 6, 2007, http://www.access.gpo.gov/su_docs/fedreg/a070606c.html , accessed June 22, 2010.

13. 2,4-D: Health Effects Division (HED) Metabolism Assessment Review Committee, (MARC) Decision Document-Revised. DP Barcodes D309452 Chemical I.D. No. 030001, Case No. 0073. Meeting date 9/3/03. October 13. 2004.

14. U. S. EPA, T. Jimerson, 2,4-D Revisions to the Product and Residue Chemistry Chapters of the Reregistration Eligibility Decision; Reregistration Case No. 0073. Chemical ID. No. 030001,; DP Barcose No. D309450 and D309451, October 12, 2004.

15. US EPA Electronic Code of Federal Regulations, 180.142; 2,4-D, tolerances for residues, http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=44f7335e7b9507a314e2aadfd0c40fa4&rgn=div8&view=text&node=40:23.0.1.1.28.3.19.20&idno=40 , accessed June 18, 2010.

16. United Nations Comtrade Database, DESA/UNSD, http://comtrade.un.org/db/ , accessed June 2010.

17. T. C. Blewett, Dow AgroSciences Regulatory, personal communication, based on internal inquiry of DAS Global Regulatory personnel and review of available posted information, April 2010.

18. IUCLID Dataset, 2000. 2,4-dichlorophenol, CAS No. 120-83-2, ecb.jrc.ec.europa.eu/iuclid-datasheet/120832.pdf , accessed June 22, 2010.

19. NTP, 1989. Technical report on the toxicology and carcinogenesis studies of 2,4-dichlorophenol in F344/N rats and B6C3F1 mice (feed studies). Technical Report Series 353.

20. Fiskesjo,G. (1988) 2,4-Dichlorophenol and MCPA in a V79 Test, ATLA. Altern Lab Anim 15: 245–250.

21. Aoyama H, Hojo H, Takahashi KL, Shimizu N, Araki M, Harigae M, Tanaka N, Shirasaka N, Kuwahara M, Nakashima N, Yamamoto E, Saka M and Teramoto S. 2005. A Two-generation reproductive toxicity study of 2,4-dichlorphenol in rats. J Toxicol Sci 30: 59-78.

22. Rodwell DE, Wilson RD, Nemec MD and Mercieca MD (1989). Teratogenic assessment of 2,4-dichlorophenol in Fischer 344 rats. Fund Appl Toxicol, 13: 635-640.

23. M. Ma, Y. A. Adelfinskaya, A Nature of Residue Study with [14C]-2,4-D DMA Applied to AAD-1 Corn (Event 278), Report 090058, Internal Report of Dow AgroSciences, LLC, July 9, 2010.

24. S. L. Rotondaro and J. L. Balcer, A Nature of Residue Study with [14C]-2,4-D DMA Applied to AAD-1 Corn, Report 080058, Internal Report of Dow AgroSciences, LLC, May 4, 2010.

25. J. F. Culligan, Magnitude of Residue of 2,4-D and Quizalofop-P-ehtyl in/on Herbicide Tolerant Field Corn Containing the Aryloxyalkanoate Dioxygenase-1 (AAD-1) Gene, Protocol ARA-09-15-10, Report No 090052, Internal Report of Dow AgroSciences, LLC, October 2010.

26. J. F. Culligan, B. M. Wendelburg, Analytical Summary for Magnitude of Residue of 2,4-D and Quizalofop-P-ehtyl in/on Herbicide Tolerant Field Corn Containing the Aryloxyalkanoate Dioxygenase-1 (AAD-1) Gene, Protocol ARA-09-15-10, Internal Report of Dow AgroSciences, LLC, October 4, 2010.

27. U. S. EPA, HED SOP 99.6, Classification of Food Forms with Respect to level of Blending, August 20, 1999.

28. US, EPA, HED memo of August 2008, Revisions of Feedstuffs in Table 1 of OPPTS Test Guideline 860.1000 and Guidance on Constructing Maximum Reasonably Balanced Diets (MRBD).

29. Novigen Sciences, Inc. DEEM Dietary Exposure Evaluation Model Users Manual, May 2000, (Page 4-5).

30. U. S. EPA, 2,4-D Revised Acute and Chronic Dietary Exposure Assessments for the Reregistration Eligibility Decision, D287661, EPA-HQ-OPP-2004-0167-0087, http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064800b999a , October 13, 2004, accessed June 21, 2010.

31. United States Department of Agriculture, USDA, Pesticide Data Program, PDP-Download Data/Reports Webpage, 1999 and 1998 Summary reports.

Table 1. Summary of 2,4-D and DCP Residue in Several Metabolism Studies

Study	Animal	Dietary Dose 2,4-D (ppm)	Residue Matrix	2,4-D residue (ppm)	DCP residue (ppm)
Ref (7)	Lactating Goat	483			
			Milk	0.095	0.010 ppm
			Liver	0.046	- -
			Kidney	0.773	- -
			Fat	0.040	0.020
			Muscle	0.014	- -
Ref (8)	Sheep	2000	Muscle	0.06	<0.05
			Kidney	5.8	0.16
			Liver	0.45	0.26
			Fat	0.07	<0.05
Ref (9)	Beef Calves	2000	Muscle	<0.05	<0.05
			Kidney	7.6	0.8
			Liver	0.4	<0.05
			Fat	0.13	<0.05
Ref (11)	Dairy Cows	1000	Milk	0.1	<0.05
Ref (10)	Sheep (alkaline)	2000	Liver	0.98	0.16
			Kidney	9.17	0.26
	Cattle (alkaline)	2000	Liver	0.23	0.31
			Kidney	10.9	1.06

Table 2. Summary of 2,4-D and DCP Toxicology Data

	2,4-D[a]	**DCP**[b]
Acute oral	866-2322 mg/kg	580-4000 mg/kg
Repeat dose (NOAEL)	15 mg/kg/day	400 mg/kg/day
Genotoxicity	Not Genotoxic	Weight of Evidence- not Genotoxic
Reproductive (NOAEL/NOEL)	Systemic: 5 mg/kg Maternal: 20 mg/kg Offspring: 5 mg/kg	Systemic: 33.4 mg/kg Maternal: 134 mg/kg Offspring: 134 mg/kg
Developmental (NOAEL/NOEL)	Parent: 25 mg/kg Offspring:75mg/kg	Parent: 200 mg/kg Offspring: 750 mg/kg
Carcinogenicity	Not carcinogenic	Not carcinogenic

[a]2,4-D RED, 2005 (5)
[b]IUCLID Dataset, 2000 (18) and Aoyama, 2005 (21)

Table 3. Summary of Residue Results of AAD-1 corn MOR Study

Sample		**2,4-D (ppm)**					**DCP (ppm)**			
Type	**PHI**	**min**	**max**	**HAFT**	**mean[a]**	**EPA Tol[b] (ppm)**	**min**	**max**	**HAFT**	**mean**
Forage	40	ND	2.988	2.612	0.641	6	ND	3.997	3.056	1.032
Forage	~60	ND	4.373	3.124	0.697	6	*0.004*	5.007	3.899	0.876
Grain[c]	90	ND	*0.004*	*0.004*	*0.0002*	0.05	ND	0.003	*0.0017*	<0.0001
Grain[d]	90	ND	0.067	0.034	0.0015	0.05	ND	0.003	*0.0017*	<0.0001
Stover	90	ND	8.920	8.076	1.349	50	ND	5.842	5.377	0.911
Aspir. Grain @ 2X	90	ND	0.021	-		40	ND	*0.006*		0.003
Starch	90	ND	ND	ND			ND	ND	ND	
Oil	90	ND	ND	ND			ND	ND	ND	
Flour	90	ND	ND	ND			ND	ND	ND	
Meal	90	ND	ND	ND			ND	ND	ND	
Grits	90	ND	ND	ND			ND	ND	ND	

[a]Mean grain values reported to 4 digits to emphasize mean values relationship to ½ LOD of 0.0015 ppm.
[b]Tol = Tolerance.

[c]without 2,4-D residue outlier.

[d]with 2,4-D residue outlier.

Table 4. Revised Animal Burdens based on MRBD and Potential AAD-1 Impact for Animal Feeds for US Meat and Milk Tolerances in Cattle

	Feed	**Type**	**DM**	**% Diet**	**Feed Tolerance (ppm)**	**MRBD Contribution (ppm)**	**MTDB RED value (ppm)**
Dairy							
Grass	forage	R	25	45	360	648	
Barley	grain	CC	88	45	2	1.02	
Alfalfa	meal	PC	89	10	0.2	0.02	
				100	**New Total ->**	**649.1**	**874**
Beef							
Grass	Hay	R	88	15	300	51.14	
Grain	aspirated fractions	CC	85	5	40	2.35	
Barley	Grain	CC	88	50	2	1.14	
Potato	processed waste	CC	15	*25*	0.4	0.67	
Soybean	Seed	PC	89	5	0.02	.001	
				100	**New Total ->**	55.3	874

DM = dry matter

Table 5. Revised Animal Burdens based on MRBD and Potential AAD-1 Impact for Animal Feeds for US Meat and Milk Tolerances in Poultry and Swine

	Feed	**Type**	**% Diet**	**Tolerance (ppm)**	**MRBD Contribution (ppm)**	**MTDB RED value (ppm)**
Poultry						
Oat, barley, wheat	Grain	CC	75	2	1.5	
Alfalfa	meal	PC	5	0.2	0.01	
Pea, field	seed	PC	20	0.05	0.01	
			100	New Total ->	1.5	1.6
Swine						
Barley	grain	CC	20	2	0.4	
Millet	grain	CC	20	2	0.4	
Sorghum, grain	grain	CC	*35*	0.2	0.07	
Alfalfa	meal	PC	5	0.2	0.01	
Pea, field	seed	PC	*10*	0.05	0.005	
			100	New Total ->	1.1	1.6

Table 6. Potential Corn Contributions to Animal MRBD based on Current 2,4-D Tolerances

Corn	**Type**	**DM**	**% Beef Diet**	**% Dairy Diet**	**% Poultry Diet**	**% Swine Diet**	**US 2,4-D Tolerance (ppm)**	**Beef diet (ppm)**	**Dairy Diet (ppm)**	**Poultry Diet (ppm)**	**Swine Diet (ppm)**
grain	CC	88	70	40	75	85	**0.05**	0.040	0.0227	0.0375	0.0425
forage/ silage	R	40	15	45	Nu	Nu	**6**	2.25	6.750	-	-
stover	R	83	15	15	Nu	Nu	**50**	9.036	9.036	-	-
			100	100	75	85		**11.326**	**15.809**	0.0375	0.0425

Table 7. Potential Corn Contributions to Animal MRBD based on DCP

Corn	**Type**	**DM**	**% Beef Diet**	**% Dairy Diet**	**% Poultry Diet**	**% Swine Diet**	**DCP (ppm)**	**Type**	**Beef diet (ppm)**	**Dairy Diet (ppm)**	**Poultry Diet (ppm)**	**Swine Diet (ppm)**
grain	CC	88	70	40	75	85	***0.0001***	average	-	0.0001	<0.0001	<0.0001
forage/ silage	R	40	15	45	Nu	Nu	**3.899**	HAFT	1.462	4.387	-	-
stover	R	83	15	15	Nu	Nu	**5.377**	HAFT	0.972	0.972	-	-
			100	100	75	85			**2.434**	**5.359**	<0.0001	<0.0001

Table 8. Relative Contributions of AAD-1 DCP to 2,4-D Animal MRBD

	2,4-D RED burden (ppm)	2,4-D MRBD (ppm)	AAD-1 DCP dietary risk contribution[a] (ppm)	**AAD-1 corn MRDB (%)**
Dairy	874	**649**	**5.359**	**0.83%**
Beef	874	55	2.434	**4.43%**
Poultry	1.6	1.53	<0.0001	**<0.01%**
Swine	1.6	1.1	<0.0001	**<0.01%**

[a] from sums in Table 7

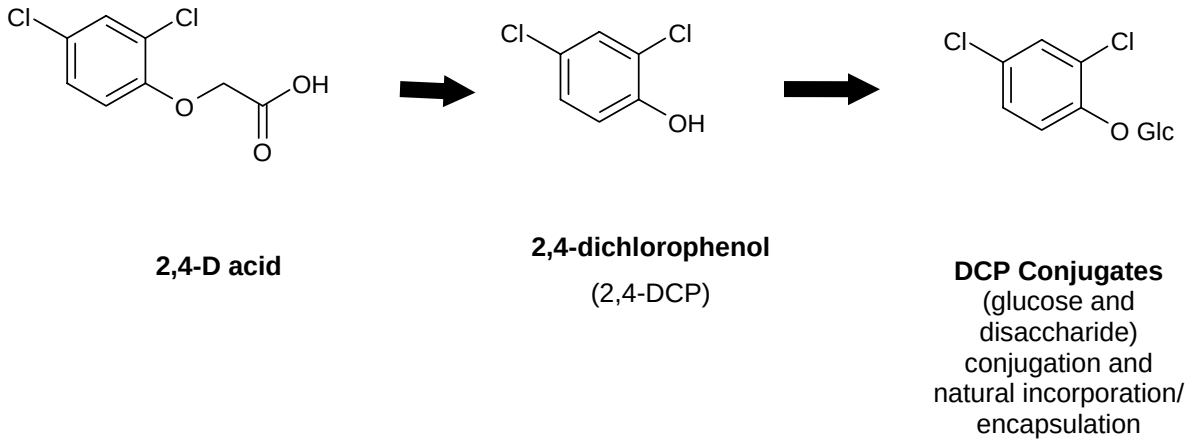

Figure 1. 2,4-D Metabolic Pathway in Plants

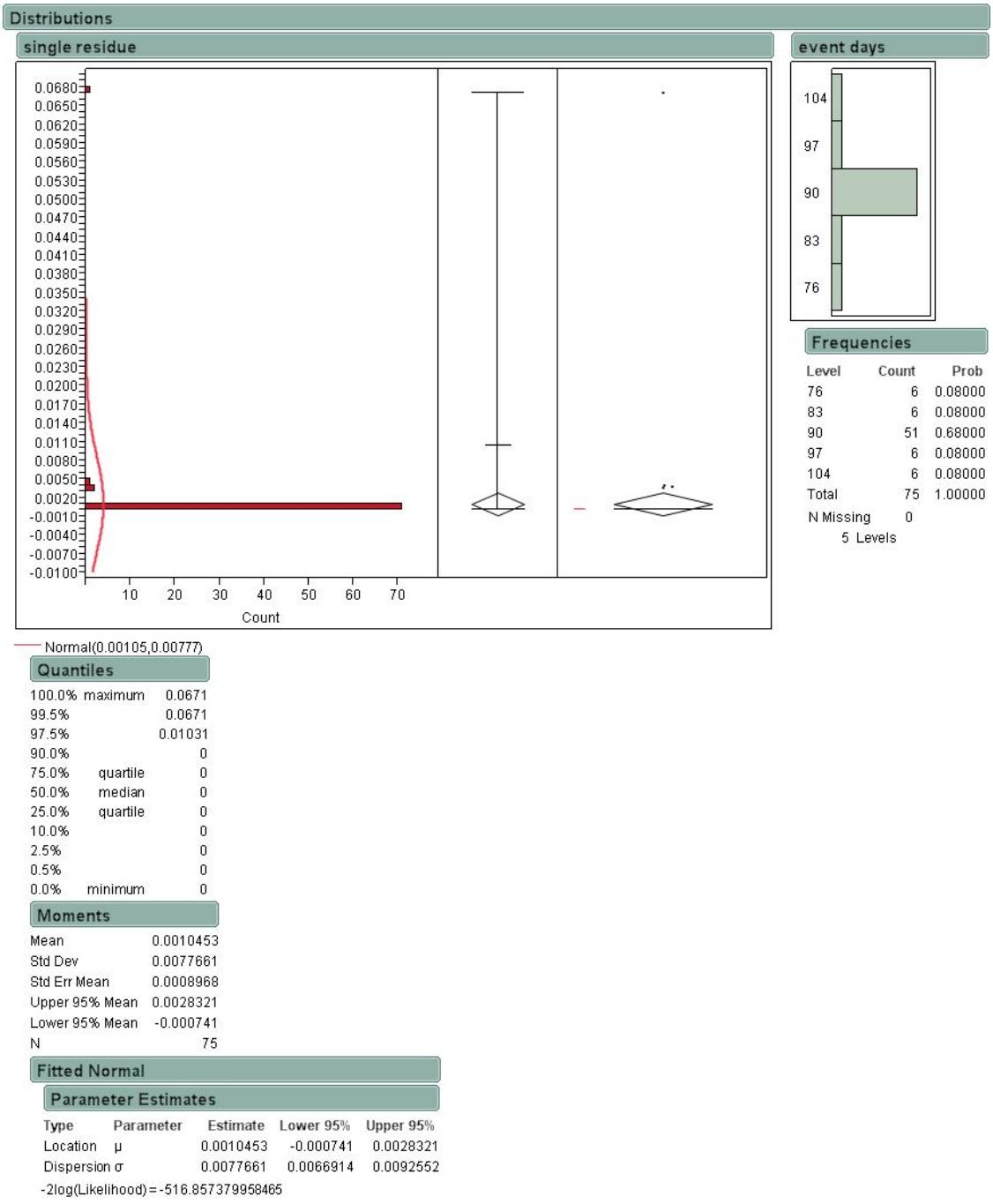

Figure 2. Distribution of Observed 2,4-D Residue in Grain Samples

Appendix A—International MRLs for 2,4-D in Corn

The following MRL values are included for comparative purposes only. Dow AgroSciences does not establish MRLs, but instead provides data to regulatory agencies which establish the values. The information is presented as "the best of our knowledge" and is current as of June 2010. The sources for the national values listed are included in the table.

Country	**Commodity Listing**	**MRL (ppm)**	**Web link to official source**
Australia	Cereal Grains	0.2	http://www.apvma.gov.au/residues/docs/table01_april_2010.pdf
CODEX	Maize	0.05	http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp
EU	Maize	0.05	http://ec.europa.eu/sanco_pesticides/public/index.cfm
Japan	Corn (maize, including pop corn and sweet corn)	0.05	http://www.mhlw.go.jp/english/topics/foodsafety/positivelist060228/index.html
USA	Corn, field, grain	0.05	http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr180_main_02.tpl
Argentina	Corn	0.05	http://www.homologa.com/
Dem. People's Rep. of Korea	Corn	0.05	http://eng.kfda.go.kr/index.php
India	Foodgrains	0.01	http://www.mohfw.nic.in/pfa.htm
Russia	Maize	0.05	http://ec.europa.eu/food/international/trade/ru_requirements_MRLs_pesticides_en.htm
Thailand	Maize	0.05	http://www.usdathailand.org/upload/TH8176%20Pesticide%20Residue%20MRLs.pdf
Chile	Corn	0.05	http://www.homologa.com/
Switzerland	Céréales	0.05	http://www.admin.ch/ch/f/rs/c817_021_23.html
Canada		0.1	*Default Value*
New Zealand		0.1	*Import Default*
Brazil	Milho	0.2	http://www.anvisa.gov.br/toxicologia/monografias/index.htm
Singapore	other cereal grains	0.2	http://www.ava.gov.sg/Legislation/ListOfLegislation/
Mexico	Corn	0.5	http://www.homologa.com/
South Africa	maize	0.5	http://www.doh.gov.za/search/index.html