5.9 DICAMBA (240)

TOXICOLOGY

Dicamba is the International Organization for Standardization (ISO)–approved name for 3,6-dichloro-2-methoxybenzoic acid (International Union of Pure and Applied Chemistry [IUPAC]) and has the Chemical Abstracts Service (CAS) No. 1918-00-9. Dicamba is a benzoic acid auxin herbicide, mimicking the action of indolyl acetic acid in regulating plant growth.

Dicamba has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues (CCPR).

All pivotal studies contained certificates of compliance with good laboratory practice (GLP).

Biochemical aspects

The absorption, distribution, metabolism and excretion of dicamba after oral dosing were investigated in several studies in rats and one study using mice, rabbits and dogs. Generally, there were no differences in the toxicokinetics of dicamba between species and sexes. Furthermore, it was shown that the anionic counter-ion of dicamba salts did not influence the absorption, metabolism or elimination of dicamba. Dicamba was rapidly absorbed, with peak levels occurring within the first hour after administration. The absorption was not saturated at doses up to 1000 mg/kg body weight (bw). Between 2 and 4 h after the first absorption peak, a second peak was observed, indicative of enterohepatic recirculation of dicamba. At doses greater than 125 mg/kg bw, the elimination half-life of dicamba equivalents increased, and the area under the curve (AUC) increased disproportionately with dose, indicating saturation of elimination at higher doses. Only 3% of a low or high dose (0.5 or 200 mg/kg bw) was found in the tissues 4 h post-dosing, with highest residues in the kidneys, plasma and uterus. After 7 days, only 0.2% of the administered dose was found in the tissues. More than 95% of the administered dose is excreted in the urine, with less than 5% in the faeces. Excretion by expired air is negligible. In urine and faeces, more than 90% of the radioactivity found was accounted for by unchanged dicamba. In urine, very low amounts of glucuronidated dicamba (M1), 3,6-dichlorosalicylic acid (DCSA; NOA 414746), 5-hydroxy-dicamba (NOA 405873) and M2 (NOA 414746) were found. In the liver and kidneys, 84–91% of total radioactive residues was identified as dicamba. In summary, dicamba is poorly metabolized, and the pathways involved include demethylation, hydroxylation and glucuronic acid conjugation.

Toxicological data

Dicamba is of low acute toxicity. The lowest oral median lethal dose (LD_{50}) was approximately 1600 mg/kg bw in female rats. By dermal application, the LD_{50} was greater than 2000 mg/kg bw, and the median lethal concentration (LC_{50}) in an inhalation study was greater than 9.6 mg/L. Dicamba was only slightly irritating to the skin but severely irritating to the eye. Dicamba did not show skin sensitizing potential.

In repeated-dose studies in rats and dogs, the mostly mild effects observed included lower body weight gains, haematological and clinical chemistry effects and clinical signs of toxicity.

In a 13-week rat feeding study with dietary concentrations up to 12 000 ppm, reduced activity, lower body weight development and reduced food consumption were observed. Animals had significantly lower platelet counts and partial thromboplastin times; females also had reduced red cell parameters and increased white blood cell and lymphocyte counts, and clinical chemistry parameters were changed. After a recovery period, most of the haematological and clinical chemistry parameters were similar to those in control animals. Relative liver weights were statistically significantly increased. Histological findings were restricted to high-dose females, which showed reversible

centrilobular hepatocyte hypertrophy and hepatocellular pigmentation. The no-observed-adverse-effect level (NOAEL) was 6000 ppm (equal to 479.3 mg/kg bw per day), based on haematological and biochemical effects at 12 000 ppm. In a 13-week dog study with administration in capsules of doses up to 300 mg/kg bw per day, including a high-dose recovery group, behavioural changes (i.e., ataxia, stiff gait and sporadic transient collapses approximately 2 h after dosing) at 300 mg/kg bw per day were observed, and body weight gain was decreased by 26% in males and by 44% in females. In the high-dose recovery group, body weight development was similar to that in the controls. Red blood cell parameters were reduced and the partial thromboplastin time was slightly elevated in males and females receiving 300 mg/kg bw per day. These effects were partially reversible within 4 weeks. The NOAEL in this study was 50 mg/kg bw per day, based on behavioural effects at 300 mg/kg bw per day. In a 52-week feeding study in dogs with dietary concentrations up to 2500 ppm, animals in the 500 ppm and 2500 ppm groups showed initially lower food consumption and lower body weight gain. This effect was transient and is not considered to be adverse. The NOAEL was 2500 ppm (equal to 52 mg/kg bw per day), the highest dose tested.

In a 24-month feeding study (89 weeks for males) in mice with dietary concentrations up to 3000 ppm, the onset of mortality was early in the study, and the overall mortality was increased in males. At 3000 ppm, body weights in females were lower from week 25 onwards. The NOAEL was 1000 ppm (equal to 108 mg/kg bw per day), based on reduced body weight gain in females at the highest dose.

Dicamba was not carcinogenic in mice.

A carcinogenicity study in rats with dietary concentrations up to 2500 ppm was considered adequate to assess carcinogenicity at 104 weeks, although survival was low at study termination (week 117). The incidences of mixed malignant lymphoma (8% versus 0% in all other groups) and thyroid C-cell carcinoma were increased in the high dose group males, although not statistically significantly. Although the incidence of malignant lymphoma was higher than the historical control range of 0–1.8%, it was at the upper bound of the historical control ranges aggregated for all types of malignant lymphoreticular lymphoma (0–8.6% and 0–8.4% in another historical control group data set). There was no increase in the incidence of C-cell hyperplasia or adenoma, which are part of the progression to carcinoma. The NOAEL for general toxicity was 2500 ppm (equal to 107 mg/kg bw per day), the highest dose tested.

Dicamba was an equivocal carcinogen in rats.

The potential genotoxicity of dicamba was tested in an adequate battery of in vitro and in vivo studies, providing no evidence of genotoxic potential.

The Meeting concluded that dicamba was unlikely to be genotoxic.

On the basis of the absence of genotoxicity and the absence of carcinogenicity in mice and the fact that an equivocal increase in the incidences of lymphoid tumours and of thyroid C-cell carcinoma in male rats occurred only at the highest dose, the Meeting concluded that dicamba is unlikely to be carcinogenic at human dietary exposure levels.

In a two-generation study of reproductive toxicity in rats at dietary concentrations up to 5000 ppm, high-dose females showed increased body tone and slow righting reflex, and high-dose F_1 and F_2 pups had lower body weights throughout the lactation phase. At weaning, their body weights were lower by more than 20%. Thereafter, body weight gain was not affected. At 1500 ppm, pup weights were also slightly reduced, attaining statistical significance at several time points in the lactation phase. There were no effects on mating performance or pregnancy at any dose level. At the high dose, balano-preputial separation was delayed statistically significantly (45.6 days versus 43.7 days in controls). The NOAEL for parental toxicity was 1500 ppm (equal to 105 mg/kg bw per day), based on behavioural effects at 5000 ppm. The NOAEL for reproductive toxicity was 5000 ppm (equal to 347 mg/kg bw per day), the highest dose tested. The NOAEL for effects on postnatal development was 500 ppm (equal to 35.1 mg/kg bw per day), based on reduced pup body weights.

In a study on developmental toxicity in rats at dose levels up to 400 mg/kg bw per day, 3 of 25 females died on treatment day 2 or 3. The mean body weight on gestation day (GD) 20 was lower (by 8%) in high-dose animals. Food consumption was reduced by approximately 20%, and animals showed behavioural changes, such as ataxia and stiffening of the body. The body weights of high-dose fetuses were statistically not significantly reduced by 6%. There were no treatment-related skeletal anomalies. The maternal NOAEL was 160 mg/kg bw per day, based on mortality and behavioural changes at 400 mg/kg bw per day, and the developmental NOAEL was 400 mg/kg bw per day, the highest dose tested.

In a study on developmental toxicity in rabbits at dose levels up to 300 mg/kg bw per day, one 150 mg/kg bw per day doe aborted on GD 22, and four does in the high dose group aborted between GD 19 and GD 24. All animals aborting showed body weight loss accompanied by reduced food consumption and ataxia. On necropsy, no lesions were observed. Generally, the mid- and high-dose dams showed decreased motor activity and ataxia, and the high-dose animals also had rales, laboured breathing and impaired righting reflex. These clinical signs were observed first on GD 9. The body weights and the food consumption of high-dose dams were reduced. There were no effects of treatment on the litter data. The NOAEL for dams was 30 mg/kg bw per day, based on behavioural changes at 150 mg/kg bw per day. The developmental NOAEL was 300 mg/kg bw per day, the highest dose tested.

The Meeting concluded that dicamba was not teratogenic.

In an acute neurotoxicity study in rats at doses ranging from 300 to 1200 mg/kg bw, nonspecific and transient neurobehavioural effects were apparent within 1.5 h after dosing in all dose groups, with a dose-dependent incidence and severity of rigidity.

In a 13-week rat feeding study of neurotoxicity with dietary concentrations up to 12 000 ppm (equal to 767.9 mg/kg bw per day), no behavioural or histological evidence for neurotoxicity was observed.

Toxicological studies for three metabolites were submitted. DCSA and 3,6-dichlorogentisic acid (DCGA) have been identified as metabolites of dicamba in soya beans, sugarcane, wheat and cotton and are also environmental metabolites of dicamba. DCSA has been identified in rats, cows, goats and hens, and 5-hydroxy-dicamba was found in rats.

In a poorly described 13-week feeding study in rats and dogs, 5-hydroxy-dicamba showed no toxicity up to 250 ppm (equivalent to 25 mg/kg bw per day in rats and 6.25 mg/kg bw per day in dogs), the highest dietary concentration tested. 5-Hydroxy-dicamba gave positive results in mouse lymphoma assays and in a Chinese hamster ovary chromosomal aberration test at cytotoxic levels, in the absence and presence of metabolic activation (S9). 5-Hydroxy-dicamba was negative in a mouse micronucleus test.

DCSA showed pharmacokinetic behaviour very similar to that of the parent dicamba and was excreted mainly unchanged and to a minor extent as DCSA carboxyl glucuronide and DCSA phenolic glucuronide (M2, also identified as a rat metabolite of dicamba). In a 13-week feeding study in rats at dietary DCSA concentrations up to 12 000 ppm, reduced body weight gain and haematological and clinical chemistry effects were observed at 6000 ppm and 12 000 ppm. The NOAEL was 3000 ppm (equal to 195 mg/kg bw per day), based on reduced body weight gain at 6000 ppm. In a 13-week study in dogs administered up to 150 mg/kg bw per day by capsule, the NOAEL was 50 mg/kg bw per day, based on reduced (by 11%) body weight gain and liver effects at 150 mg/kg bw per day. In a 52-week feeding study in rats, the NOAEL was 3000 ppm (equal to 171.2 mg/kg bw per day), the highest dose tested. In a two-generation study in rats at dietary concentrations of DCSA up to 5000 ppm, the NOAEL for parental toxicity was 500 ppm (equal to 37 mg/kg bw per day), based on lower body weight gain and reduced food consumption in both sexes at 5000 ppm. The NOAEL for offspring toxicity was 500 ppm (equal to 37 mg/kg bw per day), based on severe toxicity, including mortality, in pups during lactation. The NOAEL for reproductive performance was 5000 ppm (equal to 323 mg/kg bw per day), the highest dose tested. In a pilot and a definitive rat developmental study

on DCSA, the overall NOAEL for maternal and developmental toxicity was 100 mg/kg bw per day, based on severe dam toxicity, including mortality and lower fetal body weight, at 200 mg/kg bw per day. In a pilot and a definitive rabbit developmental study on DCSA, the overall NOAEL for maternal toxicity was 30 mg/kg bw per day, based on reduced food consumption and lower body weight gain at 65 mg/kg bw per day. The NOAEL for developmental toxicity was 65 mg/kg bw per day, the highest dose tested. In genotoxicity studies, including a mouse micronucleus test, DCSA was negative. However, in human peripheral lymphocytes, DCSA increased the number of cells with chromosomal aberrations in the presence and absence of metabolic activation by S9 after 3 or 22 h of exposure at cytotoxic levels.

The metabolite DCGA was evaluated in a 4-week feeding study in rats at dietary concentrations up to 12 000 ppm. The NOAEL was 6000 ppm (equal to 474 mg/kg bw per day), based on reduced body weight gain and lower lymphocyte counts in animals at 12 000 ppm. In a pilot rat developmental study with doses up to 1000 mg/kg bw per day, the maternal NOAEL was 50 mg/kg bw per day, based on increased incidences of rales at 200 mg/kg bw per day, and the developmental NOAEL was 1000 mg/kg bw per day, the highest dose tested. DCGA was negative in an Ames and a rat chromosomal aberration test.

It was concluded that DCSA and DCGA have toxicity similar to or lower than that of dicamba. Based on available data, 5-hydroxy-dicamba appears to be of lower toxicity than the parent.

The Meeting concluded that the existing database on dicamba was adequate to characterize the potential hazard to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.3 mg/kg bw on the basis of a NOAEL of 30 mg/kg bw per day in a rabbit developmental toxicity study, based on maternal toxicity (behavioural changes) at 150 mg/kg bw per day. A safety factor of 100 was applied. The ADI is supported by a postnatal developmental NOAEL of 35.1 mg/kg bw per day in the rat multigeneration study, on the basis of reduced pup body weights at 105 mg/kg bw per day. This ADI would also be protective against the equivocal increase in the incidences of malignant lymphoma and thyroid parafollicular cell carcinoma in male rats at 107 mg/kg bw per day.

The Meeting established an acute reference dose (ARfD) of 0.5 mg/kg bw based on a NOAEL of 50 mg/kg bw per day in the 13-week dog study, based on behavioural effects observed shortly after dosing at 300 mg/kg bw per day. A safety factor of 100 was applied.

The behavioural effects seen in a study on developmental toxicity in rabbits at dose levels of 150 mg/kg bw per day and above are not considered to be an adequate basis for an ARfD because the clinical signs were observed first after four applied doses.

A toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	1000 ppm, equal to 108 mg/kg bw per day	3000 ppm, equal to 358 mg/kg bw per day
		Carcinogenicity	3000 ppm, equal to 358 mg/kg bw per day[b]	—

Species	Study	Effect	NOAEL	LOAEL
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	2500 ppm, equal to 107 mg/kg bw per day[b]	—
		Carcinogenicity	250 ppm, equal to 11 mg/kg bw per day	2500 ppm, equal to 107 mg/kg bw per day[b]
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	5000 ppm, equal to 347 mg/kg bw per day[b]	—
		Parental toxicity	1500 ppm, equal to 105 mg/kg bw per day	5000 ppm, equal to 347 mg/kg bw per day
		Offspring toxicity	500 ppm, equal to 35.1 mg/kg bw per day	1500 ppm, equal to 105 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	160 mg/kg bw per day	400 mg/kg bw per day
		Embryo and fetal toxicity	400 mg/kg bw per day[b]	—
Rabbit	Developmental toxicity study[d]	Maternal toxicity	30 mg/kg bw per day	150 mg/kg bw per day
		Embryo and fetal toxicity	300 mg/kg bw per day[b]	—
Dog	Thirteen-week study of toxicity[d]	Toxicity	50 mg/kg bw per day	300 mg/kg bw per day
	One-year study of toxicity[a]	Toxicity	2500 ppm, equal to 52 mg/kg bw per day[b]	—

[a] Dietary administration.

[b] Highest dose tested.

[c] Equivocal increase in the incidences of malignant lymphoma and thyroid parafollicular cell carcinoma in male rats.

[d] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.3 mg/kg bw

Estimate of acute reference dose

0.5 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to dicamba

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, > 90% within 24 h
Distribution	Extensive, highest levels in kidneys, plasma and uterus
Potential for accumulation	None
Rate and extent of excretion	Rapid, close to 100% within 48 h, mainly via urine

Metabolism in animals	Poorly metabolized, primarily via demethylation, hydroxylation and glucuronidation
Toxicologically significant compounds in animals, plants and the environment	Dicamba, DCSA, DCGA
Acute toxicity	
Rat, LD_{50}, oral	1600 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 9.6 mg/L
Rabbit, dermal irritation	Slightly irritating
Rabbit, ocular irritation	Severely irritating
Guinea-pig, dermal sensitization	Not a sensitizer (Magnusson and Kligman test)
Short-term studies of toxicity	
Target/critical effect	Body weight reduction, haematology and clinical chemistry (dogs)
Lowest relevant oral NOAEL	50 mg/kg bw per day (dogs)
Lowest relevant dermal NOAEL	2500 mg/kg bw per day, the highest dose tested (rabbits)
Lowest relevant inhalation NOAEL	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body weight gain
Lowest relevant NOAEL	1000 ppm, equal to 108 mg/kg bw per day (mice)
Carcinogenicity	Equivocal increase in malignant lymphoma and thyroid C-cell carcinoma (rats) at 2500 ppm (equal to 107 mg/kg bw per day); unlikely to be carcinogenic at human dietary exposure levels
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects, offspring toxicity (rats)
Lowest relevant reproductive NOAEL	347 mg/kg bw per day, the highest dose tested (rats); 30 mg/kg bw per day (maternal toxicity in rabbits)
Lowest relevant offspring NOAEL	35.1 mg/kg bw per day (rats)
Developmental target/critical effect	No developmental effects (rats, rabbits)
Lowest relevant developmental NOAEL	300 mg/kg bw per day (rabbits), the highest dose tested
Neurotoxicity/delayed neurotoxicity	
	Not neurotoxic
Other toxicological studies	
	Metabolism, pharmacokinetic, toxicity and genotoxicity studies with metabolites
Medical data	
	No data

Summary

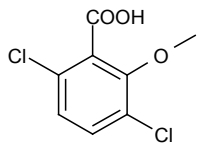
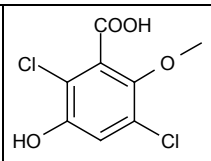
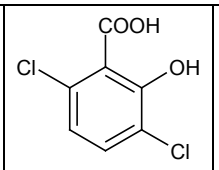
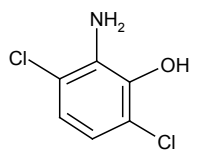
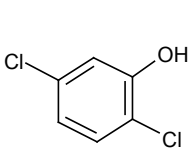
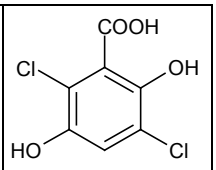
	Value	Study	Safety factor
ADI	0–0.3 mg/kg bw	Developmental toxicity study in rabbit	100
ARfD	0.5 mg/kg bw	Thirteen-week study of toxicity in dog	100

RESIDUE AND ANALYTICAL ASPECTS

Dicamba, a systemic broad-spectrum herbicide, is used in a variety of crops. Its mode of action is similar to that of endogenous auxin (IAA) and other auxin-type herbicides and appears to involve cell wall plasticity and nucleic acid metabolism.

It was identified as a priority new compound at the Forty-second Session of the CCPR in 2009 (ALINORM 09/30/24, para. 193) for evaluation for the first time by the 2010 JMPR. The Meeting received information on physical and chemical properties, animal and plant metabolism, environmental fate, analytical methods, storage stability, use patterns, supervised trials, processing and farm animal feeding.

The structures of dicamba, along with those of metabolites referred to in this appraisal, are shown below.

					
Dicamba	5-OH Dicamba	DCSA	2A36DCP	DCP	DCGA

Animal metabolism

The Meeting received information on the fate of orally-dosed dicamba in lactating goats and cow, and laying hens.

When ^{14}C-dicamba, uniformly labelled with ^{14}C in the phenyl ring, was administered orally at a dose equivalent to a dietary concentration of 10 ppm to a lactating goat daily for four consecutive days, 83%, 8.5% and 0.019% of the total administered radioactivity (TAR) was eliminated via urine, faeces and milk respectively, indicating that the majority of administered radioactivity was rapidly incorporated and excreted in urine. Total recovered radioactivity was 93% of the TAR.

Radioactivity in milk was low throughout the 4 day study period with the cumulative radioactivity of only 0.019% of the TAR. The maximum radioactive residues were 0.002 mg/kg in dicamba equivalents.

Total radioactive residues (TRR) in tissues after sacrifice (24 hours after the last dose) were also low at 0.014 mg/kg (0.023% of TAR), 0.054 mg/kg (0.014% of TAR), 0.011 mg/kg (0.033% of TAR) and 0.004 mg/kg (0.12% of TAR) in parent equivalents in liver, kidney, fat and muscle respectively.

Dicamba was rapidly incorporated into body of lactating goat after oral administration but poorly metabolized. The primary residue was the parent compound (63–93% of TRR) in all tissues

analysed with much smaller amounts of DCSA (1.2–12% of TRR). An unidentified metabolite was found at 0.10% of TRR.

Acid hydrolysis of unextracted radioactivity of liver, kidney and fat released 26–30% of TRR into ethyl acetate fraction which contained metabolites identical to dicamba and DCSA.

Radioactive residues in milk and muscle were not characterized due to their extremely low levels (maximum 0.002 mg/kg in milk and 0.004 mg/kg in muscle expressed in dicamba equivalents).

The result indicates that the major metabolism of dicamba involves O-demethylation to form DCSA although the parent compound was the predominant residue observed in excreta and tissues.

When [phenyl-U-^{14}C]-dicamba was administered orally at a dose equivalent to a dietary concentration of 60 ppm to a lactating cow twice a day for five consecutive days, 89%, 1.5% and 0.018% of TAR were recovered from urine, faeces and milk, respectively. This indicates that the administered radioactive carbon was rapidly incorporated and excreted into urine.

Radioactivity in milk was very low throughout the 5 day study period with the cumulative radioactivity of only 0.018% of the TAR. The maximum radioactive residues were 0.004 mg/kg in dicamba equivalents.

The TRR in tissues after sacrifice (6 hours after the last dose) were also low at 0.30 mg/kg, 2.59 mg/kg, 0.02 mg/kg and 0.03 mg/kg in parent equivalents in liver, kidney, fat and muscle respectively.

Unchanged dicamba was the major radioactive residue found in excreta (75–84% of TRR) and also tissues (51% of TRR in liver, and 70% of TRR in kidney). Much smaller amount of DCSA was found in urine (14–18% of TRR), faeces (8–13% of TRR), liver (21% of TRR) and kidney (11% of TRR). DCSA was the only detected metabolite in milk but its too low level did not allow confirmation of the structure. Radioactive residues in muscle were not characterized due to their low level.

The only other metabolites detected were DCSA glucuronide and DCP in urine but both were less than 4% of TRR.

The above result indicates that the metabolism of dicamba in cow occurs primarily through O-demethylation. It also involves conjugation of DCSA with glucuronic acid and decarboxylation of DCSA to produce DCP.

When [phenyl-U-^{14}C]-dicamba was administered orally at a dose equivalent to a dietary concentration of 10 ppm to a group of laying hens once a day for four consecutive days, 89% and 0.014% of TAR were eliminated in excreta and eggs, respectively. The total recovered radioactivity was 89% of the TAR.

The TRR in tissues after sacrifice (24 hours after the last dose) were very low at 0.0029 mg/kg (0.003% of TAR), 0.0003 mg/kg (0.004% of TAR), 0.0005 mg/kg (0.004% of TAR) and 0.0002 mg/kg (0.001% of TAR) in parent equivalents in liver, breast muscle, leg muscle and fat, respectively, showing little transfer to edible portion of chicken. The radioactive residues in egg white and yolk were also low and never exceeded 0.0035 mg/kg in dicamba equivalents or 0.001% of TAR.

Almost the entire radioactivity found in excreta was attributed to unchanged dicamba and 61% of the TRR in liver and 95% of the TRR in eggs were also attributed to dicamba. 2A36DCP was identified from liver at 36% of the TRR but not from excreta. DCSA and 5-OH dicamba were only identified from urine at 1.6% and 0.0004% of the TRR, respectively. Radioactive residues in muscle and fat were not characterized due to their extremely low level (< 0.0005 mg/kg in dicamba equivalents).

When laying hens were given [phenyl-U-^{14}C]-dicamba as a single oral dose (equivalent to 1 ppm and 100 ppm in the diet) or intravenously (equivalent to 1 ppm in the diet), radioactivity was rapidly incorporated and then eliminated in excreta (78–87% of TAR).

Soon after administration, radioactivity appeared in blood, reached the maximum only 30 min after dose, and decreased rapidly. The half-life of 1.1 hours in blood was calculated.

Only very low amounts of radioactivity were found in the tissues. A total of about 0.06% of the intravenously administered radioactivity was found in eleven tissues 24 hour after administration while 0.7–0.8% of the orally administered radioactivity (1 mg/kg bw) was found in these tissues after the same period. Most of the recovered radioactivity was found in kidney in both experiments.

Dicamba is almost only radioactive compound found in excreta and kidney (≥ 94% of TRR) with small amounts of DCSA (0–4.9%). There are a number of unidentified metabolites found but none exceeded 1.1% of TRR except in kidney one unidentified metabolite accounted for 5.8% of TRR.

Limited metabolism of dicamba was observed in ruminants and hens as the unchanged parent compound was the primary residue component in milk and all ruminant tissues as well as eggs and all avian tissues. Metabolism of dicamba appears to follow the same pathway in goat, cow, hen and rat. The metabolic pathway involves O-demethylation to give DCSA; hydroxylation to produce 5-OH dicamba; decarboxylation of DCSA to give DCP; substitution of carboxyl group of DCSA with amino group to form 2A36DCP; and conjugation of DCSA with glucuronic acid.

Plant metabolism

The Meeting received information on the fate of dicamba after foliar applications or treatment simulating foliar application of [phenyl-U-^{14}C]-dicamba on soya bean, wheat, sugar cane and cotton for which residue trial data were submitted to the present Meeting. Soya bean, wheat and cotton were treated and grown in the field while sugar cane was treated and grown in glasshouse.

[^{14}C]-Dicamba was applied at a sub-toxic rate of 5.17 µg/plant to soya bean plants (foliar application) grown on untreated soil at two different timings.

With the early podfill growth stage application, radioactivity rapidly decreased from 85% to 4.6% of the total applied radioactivity (TAR) in treated leaves in the first seven days after application. After 14 days, the total recovered radioactivity averaged 42% of the TAR, about a half of which was found in immature beans. This indicates rapid and significant incorporation and translocation from leaf to beans.

Over 64% and 94% of respective TRR were attributed to unchanged dicamba in treated leaves and immature beans 14 days after treatment. About 17% of the TRR in the leaf samples collected 14 days after treatment (DAT) was attributed to DCSA while only 0.6% of the TRR in the 14-DAT immature bean samples was DCSA. No di-hydroxylated metabolites were observed.

The result indicates that dicamba is translocated without metabolization or conjugation while at the site of application, dicamba goes through gradual O-demethylation.

With late senescent growth stage application, also rapid decline of radioactivity from 77% to 11% of TRR was observed in treated leaves 6 days after application. Only 63% of the TAR was recovered in the plant 6 days after treatment, among which 26% was found in the intact plant while another 24% was recovered from abscised leaves.

Untreated leaves, stems, roots, pods and immature beans 6 days after treatment contained similar radioactivity. Their radioactivity levels were similar also to those of the same tissues after early podfill stage application except immature beans. With late senescent growth stage application, there was far less translocation of dicamba to beans compared to early podfill stage application. Only 2.1% of TAR or 8% of the TRR remained in immature beans 6 days after treatment.

About 64% and 44% of respective TRR were attributed to unchanged dicamba in treated leaves (not abscised) and beans 6 days after treatment. Only 0.3% and 0.7% of the respective TRR were attributed to DCSA in 6-DAT bean sample and treated leaves (not abscised). Similarly small amounts of 5-OH dicamba and DCGA were also found in treated leaves and immature beans but neither exceeded 1.0% of TRR.

Foliar application of [^{14}C]-dicamba to spring wheat resulted in the majority of radioactivity recovered from leaves and stems and later from straw (1.1–1.9 mg/kg in dicamba equivalents). On the other hand, there was little translocation to grain at 0.056 mg/kg in dicamba equivalents.

In grain, forage and straw samples, none of free individual metabolites were > 5% of TRR and > 0.01 mg/kg, except free dicamba in grain (16% of TRR but 0.009 mg/kg). Including conjugated forms, 5-OH dicamba was the most predominant radioactive residue in straw at 3.7% of TRR and 0.70 mg/kg, and dicamba in grain as described above.

Significant amounts of radioactivity were incorporated into unextracted plant matrix constituents, such as protein, cellulose, pectin and lignin.

The above result indicates that metabolism of dicamba in wheat was extensive and includes hydroxylation at 5-position of dicamba to form 5-OH dicamba and its O-demethylation to form DCGA; O-demethylation of dicamba to form DCSA; O-demethylation of dicamba and hydroxylation at 5-position to form DCGA; and conjugation and incorporation into plant matrix constituents.

After foliar application of [^{14}C]-dicamba to 6-week old sugar cane plants grown in untreated soil at a rate of 3.06 mg/plant, dicamba was rapidly taken up by leaves with 46% of TAR recovered from plant 28 days after treatment. More than 90% of the incorporated radioactivity remained in treated leaves with about 6% TAR translocated to other parts of the plant 28 days after treatment.

Dicamba was predominant radioactive residue in treated leaves at 0 DAT (more than 90% of TRR) but decreased to less than half of TRR by 12 DAT. Over time 5-OH dicamba was formed and reached 49% of TRR by 12 DAT. At 28 DAT, the total extractable amount of 5-OH dicamba was greater than that of dicamba itself. Small amount of DCGA was also found at a total of about 2% of TRR. Amounts of unextracted radioactivity were also significant indicating incorporation of radioactivity into plant matrix. β-Glucosidase treatment released significant amount of DCSA.

Metabolism of dicamba in sugar cane seems to involve as primary pathway, hydroxylation to form 5-OH dicamba. Other pathway may include O-demethylation to form DCSA and its conjugation to form β-D-glucosides; and O-demethylation of 5-OH dicamba and hydroxylation of DCSA to form DCGA.

[^{14}C]-Dicamba was applied to cotton grown in untreated soil at a rate of 60 μg/plant, a sub-toxic rate, at the green-boll growth stage. Radioactivity in treated plants rapidly declined from 16% to 1.9% of TAR in 14 days after foliar application. On the other hand, the 14 DAT untreated leaf, stem root and boll samples contained comparable radioactivity; in particular, bolls contained 22% of TAR. This indicates significant translocation to bolls.

Among parts of the 14 DAT bolls, the majority of radioactivity (17% of TAR) is located in carpels with 2.5% and 2.6% of TAR in seed and lint respectively.

Dicamba was the predominant radioactive residue in ether fractions of treated seed (14 DAT) at 2.2% of TAR. Further analysis indicated that dicamba was poorly metabolized or not conjugated in cotton seed.

Dicamba was the predominant residue in all analysed cotton parts with very slow metabolization showing minor amounts of 5-OH dicamba.

In summary, in wheat and sugar cane, there is little translocation and dicamba was rapidly metabolized after foliar application of dicamba. In these plants, hydroxylation to form 5-OH dicamba appears to be the primary metabolic pathway. Conjugation of 5-OH dicamba is also observed.

In soya beans and cotton, which are susceptible to dicamba, metabolism of dicamba appears to be slow and limited to occur in treated leaves. However, significant translocation was observed.

Despite some differences in the rate of metabolism and translocation, there seems to be a common metabolic pathway of dicamba after its foliar application to these four plant species. The metabolism of dicamba appear to follow: hydroxylation of dicamba at the 5-position to form 5-OH dicamba; O-demethylation of 5-OH dicamba to form DCGA; O-demethylation of dicamba to form DCSA; O-demethylation of dicamba and hydroxylation to form DCGA; and conjugation of 5-OH dicamba and DCSA with glucose to form the β-D-glucosides.

Environmental fate in soil

The Meeting reviewed information on aerobic soil metabolism, aqueous photolysis and rotational crop study.

Aerobic soil metabolism

Aerobic soil metabolism studies were conducted using ^{14}C-dicamba applied to various soils and incubated under aerobic conditions at 20–25 °C. Under aerobic conditions, dicamba applied to soil was degraded very rapidly with O-demethylation, which was induced by microorganisms. DCSA was the predominant degradate in soil with its maximum level at 14–59% of the applied radioactivity. It is further degraded to 0.1–15% of the applied radioactivity at the termination of studies. A small amount of 2,5-diOH dicamba was also observed indicating possible hydroxylation of DCSA. Mineralization in the presence of microorganism was also rapid and amounting to 27–67% of the applied radioactivity at the termination of studies.

Components associated with fulvic acid were low with the maximum at 1.4–11% of applied radioactivity. However those associated with humic acid were higher with the maximum at 16–34% of the applied radioactivity.

Calculated half-life of dicamba ranged between 2.1 day and 26 days under laboratory conditions at 20–25 °C. That of DCSA ranged between 1.7 days and 45 days. These values indicate that dicamba is not persistent in soil under laboratory conditions.

Field dissipation studies with the application rate of 480 g ai/ ha confirmed fast degradation. Only the 0-10 cm soil layer contained significant amount of dicamba at the beginning of the study and the 10-20 cm and 20-30 cam soil layers contained trace amounts of dicamba. Dicamba was rapidly degraded to < 0.01 mg/kg within 21 days.

DCSA was found only in the top 0–10 cm soil layer at a maximum of 0.03 mg/kg between 6 days and 14 days after treatment. After 28 days, it also decreased to 0.01 mg/kg or less.

Dicamba and DCSA were shown to be not persistent in soil in the field.

In the other field studies with application rate of 360 g/ha, dicamba applied to soil surface decreased rapidly to < 0.01–0.29 mg/kg in the top 10 cm soil in 2–3 days after application. Within 30–60 days after treatment, dicamba decreased to 0.01 mg/kg or below.

DCSA was formed during the test period. In one study it, reached its maximum between 7 days and 15 days after application at 0.09 mg/kg and decreased thereafter to 0.02–0.05 mg/kg 120 days after application.

Calculated half-life of dicamba ranged between 1.4 and 11 days under the field condition and that for DCSA was about 10 days. These results also confirm that neither dicamba nor DCSA is persistent in soil.

Degradation pathway of dicamba in aerobic soil appears to involve O-demethylation of dicamba to form DCSA; hydroxylation of DCSA to form 2,5-diOH dicamba; hydroxylation of

dicamba to form 5-OH dicamba followed by O-demethylation to form 2,5-diOH dicamba; incorporation of further degradates into soil matrix: and mineralization.

Photolysis on dry soil

Under xenon arc (simulating 40°N latitude summer sunlight) at 25 °C, dicamba degraded slowly on dry soil surface with about 20% of dicamba photo-degraded in 30 days. Without irradiation, no significant loss of dicamba was observed in 30 days. This indicates that photolysis on soil surface by light is not regarded as an important degradation pathway for dicamba.

Residues in succeeding crops

In an outdoor confined rotation study, mustard, turnip and wheat were planted at 32, 131 and 369 days after the application of ^{14}C-dicamba at a rate of 560 g ai/ha to soil.

Only samples from rotational crops planted 32 days after soil treatment contained detectable radioactive residues. No radioactive residues were detected in samples from crops planted 131 or 369 days after soil treatment. These results indicate negligible uptake of dicamba by rotational crops from soil. Crops planted 32 days after treatment contained 0.0015 mg/kg (turnip tops) to 0.21 mg/kg (mustard tops) in dicamba equivalents. Wheat forage contained 0.033 mg/kg in dicamba equivalents.

DCSA or 5-OH dicamba was not detected in these crops from all plant back intervals.

These results indicate rapid degradation of dicamba in soil and limited uptake of dicamba into plants. Analysis of soil confirmed the rapid degradation and dissipation of both dicamba and DCSA in soil.

In another rotational crop study with plant back intervals of 214, 301 and 542 days after treatment at a rate of 2.24 kg ai/ha, similar results were observed with the maximum at 0.043 mg/kg in dicamba equivalents in turnip tops from 214 day plant back interval. Analysis of soil also showed rapid degradation and dissipation of dicamba and DCSA in soil.

In the third rotational crop study, collard greens, carrot and barley were planted 30, 120 and 365 days after treatment at a rate of 840 g ai/ha. While these crops planted 30 days after treatment contained radioactive residues at 0.026–9.5 mg/kg in dicamba equivalents, those planted 120 days after treatment contained radioactive residues at < 0.01–0.036 mg/kg and no crop planted 365 days after treatment contained detectable radioactive residues.

It is concluded that no or little dicamba residues were expected to occur in rotational crops.

Methods of analysis

Analytical methods for determination of residues of dicamba and its metabolites were developed for a wide range of matrices of plant and animal origin. In general, these methods employ homogenization, hydrolysis at 95 °C for 1.5 hours and extraction with 1N HCl, neutralization, and re-acidification, extraction with ethyl ether, methylation with diazomethane, clean-up, and analysis using GC/ECD. Confirmation was done using GC/MSD. The HCl hydrolysis process releases conjugated dicamba and its metabolites.

The methods for plant matrices were validated for each analyte at 0.01–1.0 mg/kg, and in case of pasture grass and hay at 20–100 mg/kg.

Method AM-0-766A was successfully validated at the fortification levels of 0.01–0.50 mg/kg for dicamba and DCSA in asparagus.

Method AM-069B and its better presented method AM-0691B-0297-4 were successfully validated (recovery 70–120%) at the fortification levels of 0.05–5.0 mg/kg for dicamba and 5-OH dicamba in barley grain and straw; maize grain, silage, stalk and stover; cotton seed, trash, seed hull, seed meal, crude seed oil and refined seed oil; peanut hay (green); sorghum grain and silage; soya

bean seed, forage, stalk and straw; sugar cane leaf and stalk; tomato, tomato juice, tomato pomace and tomato sauce; and wheat grain, silage, straw, bran, germ and flour. It was also validated at fortification levels of 20–100 mg/kg for dicamba and 5-OH dicamba in pasture grass and hay. However, the overall mean relative standard deviation was slightly higher than 20% (21–23%) for 5-OH dicamba in soya bean seed and forage, and wheat silage.

Method AM-0691B-0297-3 was also successfully validated for the same fortification levels as above for dicamba and 5-OH dicamba in barley grain and straw: maize forage, grain, silage and fodder; wheat grain, and pasture grass and hay. However, the relative standard deviation was slightly higher than 20% (23 and 26%) for 0.10 mg/kg 5-OH dicamba in barley grain and wheat grain.

Method AM-766A-1093-2 involving butylation with diazobutane, was validated successfully for 0.01–3 mg/kg of dicamba and 0.01–0.1 mg/kg of DCSA in asparagus.

Method AM-0941-1094-0, using butylation, rather than methylation, was successfully validated for 0.02–5.0 mg/kg of dicamba and DCSA and 5-OH dicamba in asparagus and soya bean except fortification level of 0.10 mg/kg in soya bean which showed a recovery of 63%. However, overall relative standard deviation of fortified soya bean samples were higher than 20% (25% for dicamba, 28% for DCSA and 25% for 5-OH dicamba.

Method REM 193.01 was successfully validated for the purpose of enforcement for 0.01 and 0.10 mg/kg of dicamba and 5-OH dicamba in maize, whole plant, grain and straw; rape seed; pasture and oranges.

The multiresidue methods described in the FDA PAM were tested for DCSA and 5-OH dicamba. After screening, Protocols C, A and B were tested. While GPC test resulted in recoveries of 5-OH dicamba and DCSA within acceptable range, recoveries of 5-OH dicamba and DCSA in soya bean forage through complete Protocol B were at or below 6%. From the soya bean seed, 5-OH dicamba showed 0% of recovery at all fortified levels.

The methods for animal matrices employ very similar procedures as the methods for plant matrices described above. They were validated for dicamba and DCSA at 0.01–3.0 mg/kg in bovine tissues, milk and eggs.

Method AM-0938-0994-0, using butylation with diazobutane, was successfully validated for 0.01–0.50 mg/kg of dicamba in beef fat, kidney, liver, muscle and milk except that for 0.01 mg/kg fortification to liver, the recovery was 65%. For 0.01–0.50 mg/kg of DCSA, the recoveries of around 65% were seen for beef fat and liver. On the contrary, the recovery of 140% was seen for kidney. Relatively high relative standard deviations (25–47%) were seen for fat, liver and milk. It was successfully validated for muscle. In the confirmatory trial, it was again successfully validated for 0.1–3.0 mg/kg dicamba in beef fat and liver and for 0.10–0.50 mg/kg DCSA in fat. However, in the confirmatory trial, recoveries of 0.75–3.0 mg/kg DCSA in liver were in a range of 50–56%.

Method GRM022.03A using N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide to produce tertiary butyl demethyl silyl esters was successfully validated as enforcement method for 0.01 and 0.10 mg/kg of dicamba and DCSA in eggs, milk, beef muscle, fat, liver and kidney. However, in the second validation study, the method was successfully validated only for 0.01 and 0.10 mg/kg dicamba in eggs, milk, beef muscle, liver and kidney and 0.01 and 0.10 mg/kg DCSA in muscle and liver.

In most methods, limit of quantitation was 0.01 mg/kg. For some matrices, such as asparagus, soya beans and cotton, the LOQs were higher at 0.02 mg/kg.

The methods used in cattle feeding studies were not described among the analytical methods. The method used in the 1979 studies determined dicamba and DCSA inseparably as methyl ester of dicamba using GC/ECD.

Stability of residues in stored analytical samples

Stability of dicamba and its metabolites (fortification level of 0.1–0.5 mg/kg) in homogenised asparagus, soya bean, maize and sorghum stored in deep freezer was investigated 3–36 months reflecting the sample storage periods in residue trials.

In asparagus, remaining dicamba and DCSA were 75% and 81% respectively after 104 days of frozen storage, and remaining 5-OH dicamba was 87% (unadjusted for procedural recovery) after 119 days frozen storage. In these specified time, dicamba, DCSA and 5-OH dicamba are stable in the frozen storage.

After frozen storage for 81 days, residues of dicamba and 5-OH dicamba were stable in sugar cane, and for 60 days in bagasse and final molasses with more than 95% of the original residues remaining.

After frozen storage for 3 months, residues of dicamba and 5-OH dicamba were stable in soya bean with 79% and 91% of the original residues remaining respectively. They were similarly stable in refined soya bean oil with 79% and 86% remaining respectively. However, 63% and 65% of DCSA were remaining after 3 month frozen storage in seeds and refined oil showing some degradation but procedural recoveries were also low at 68% and 71% in seeds and refined oil respectively.

Dicamba in maize grain, forage, fodder and silage was stable frozen up to 36 months. 5-OH dicamba was stable frozen for up to 36 months in maize grain and forage but up to only 3 months in fodder and silage.

Dicamba and 5-OH dicamba were stable up to 5 months in sorghum grain and up to 2 months in grain dust.

Dicamba was demonstrated to be stable frozen up to 18 months in animal tissues. DCSA was generally stable for the same period in animal tissues but, in liver and muscle, showed to be unstable beyond 3 months.

Definition of the residue

In goats, cows and hens, metabolism of dicamba was limited. The parent compound remained as major components in ruminant and avian tissues. In goats and cows, much smaller amount of DCSA was found in liver and kidney. In hen metabolism studies after oral administration of dicamba at a dose level equivalent to 10 ppm, 2A36DCP was identified from liver extract at 0.001 mg/kg (36% of TRR) while DCSA was not detected from analysed matrices, liver, kidney or eggs.

In soya bean and cotton, the predominant residue was dicamba. In soya bean much less amount of 5-OH dicamba was detected. While DCSA was not found after early podfill stage foliar treatment, it was found at a very small amount after late senescent stage foliar application.

In sugar cane, major residues were 5-OH dicamba and dicamba with a very small amount of DCGA after foliar application.

In wheat grain, forage and straw, free metabolites were all < 0.01 mg/kg. Dicamba was predominant in grain while 5-OH dicamba was the predominant residue in straw.

Sufficiently validated GC/ECD methods were available for determining the parent compound, 5-OH dicamba and DCSA in a wide range of plant commodities and animal tissues, milk and eggs.

Based on the above findings, the Meeting considered that the parent dicamba was suitable residue for enforcement.

However, as DCSA is a major metabolite in goats and cows and in the cattle feeding study DCSA was not separately determined from dicamba, the Meeting decided to include DCSA in the

residue definition for both enforcement and estimation of dietary intake for animal commodities. In many trials on crops, DCSA was not determined.

5-OH dicamba, a major metabolite in plants, is formed in significant amounts in some plant species, the Meeting decided to include this compound in the residue definition for plant commodities for estimation of dietary intake.

Dicamba has logPow of –0.5 and –1.8 at pH 5 and 7, respectively, at 25°C, indicating that dicamba is not fat-soluble. In animal metabolism studies, there was no specific residue concentration found in tissues with higher fat content.

The Meeting recommended the following residue definition for plant and animal commodities:

Definition of the residue for plant commodities (for compliance with the MRL): *Dicamba*

Definition of the residue (for estimation of dietary intake) for plant commodities: *Sum of dicamba and 5-OH dicamba expressed as dicamba*

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for animal commodities: *Sum of dicamba and DCSA*

Residue is not fat-soluble.

Results of supervised residue trials on crops

The Meeting received supervised trial data for dicamba on sweet corn, soya bean, asparagus, barley, maize (field corn), sorghum, wheat, sugar cane, cotton and pasture grasses. All trials were conducted in the USA.

For all analytes and matrices, generally the LOQ was 0.01 mg/kg unless as otherwise stated.

For summing up the total residues, if dicamba, 5-OH dicamba and DCSA were below the LOQ, the LOQ value of each was used for calculation.

Sweet corn

Nine supervised trials were conducted. Two treatments were applied side-by-side consisting of two applications of 0.14 kg ai/ha (total 0.28 kg as/ha; 50% WG) and two applications of 0.28 kg ai/ha (total 0.56 kg ai/ha; 480 g/L SL). The timings for the sequential applications were early post-emergence (12 inch tall corn plant) and mid post-emergence (24 inch tall corn plant). The LOQ was 0.02 mg/kg for dicamba and 5-OH dicamba. DCSA was not determined.

The US GAP allows one application at a rate of 0. 14 kg ai/ha with a PHI of 72 days.

The trials were conducted with 2 applications at a rate of 0.14 kg ai/ha with PHI of 21–60 days. Under this condition, that would lead to higher residues, resulting dicamba residues were < 0.02 (8) and 0.02 mg/kg.

Taking into consideration, the trial condition, the Meeting estimated a maximum residue level of 0.02 mg/kg for sweet corn.

Corresponding total residues of dicamba and 5-OH dicamba in rank order were: < 0.04 (8) and 0.04mg/kg.

The Meeting estimated an STMR and HR at 0.04 and 0.04 mg/kg.

Because residues were mostly below the limit of quantitation the NAFTA calculator was not used.

Soya bean

A total of 23 trials were conducted.

Each formulation was applied at the maximum proposed label rate according to the methods and conditions representing U.S. soybean production.

US GAP allow two different applications: application of 0.56 kg as/ha as a broadcast made to the soil surface approximately 14 prior to planting and application of 2.24 kg ai/ha applied approximately 14 days prior to harvest. The PHI is 14 days for the latter use. .

In all the trials, the second application was carried out 7 days before normal harvest, shorter than GAP PHI. In addition, the total applied rate exceeded the maximum seasonal rate. There were significant residues found in soya bean seeds but it is not possible to estimate residue levels at 14 day PHI.

The Meeting concluded that since no trial matched the GAP, no maximum residue level could be recommended.

Asparagus

US GAP allows the use of dicamba in asparagus with one application of 0.56 kg as/ha (0.56 kg ai/ha total maximum seasonal application) and PHI of one day.

A total of eight supervised field residue trials were conducted. The formulations used at each site were the dimethylamine salt (DMA^+), the diglycolamine salt (DGA^+), and the sodium salt (Na^+) of dicamba. Each formulation was applied at the maximum proposed label rate according to the methods and conditions representing U.S. asparagus production. There was no statistically significant difference in residues after application with different salt type.

Residues from trials matching the GAP were: 0.45, 0.49, 0.58, 0.78, 0.96, 1.1, 2.3 and 3.3 mg/kg.

The Meeting estimated a maximum residue level of 5 mg/kg.

Corresponding total residues of dicamba were: 0.46, 0.50, 0.59, 0.79, 0.97, 1.11, 2.34 and 3.28 mg/kg.

The Meeting estimated an STMR and HR at 0.87 mg/kg and 3.3 mg/kg.

The maximum residue level estimate derived from use of the NAFTA statistical calculator was 6 mg/kg, which differed from the estimate made by the Meeting.

Barley

US GAP allows two applications: one application of 0.14 kg as/ha immediately prior to the first joint stage and one application of 0.28 kg ai/ha. The PHI is 7 days.

A total of 11 supervised field residue trials were conducted. The dimethylamine salt (DMA^+) of dicamba was applied in five trial locations. Side by side trials with three formulations (the dimethylamine salt (DMA^+), the diglycolamine salt (DGA^+), and the sodium salt (Na^+) of dicamba) were conducted at four locations to determine the similarity of residues from the different salts. The statistical analysis indicated different salt type did not influence residue levels.

Residues of dicamba from trials matching US GAP were: 0.78, 1.1, 1.1, 1.5, 1.6, 1.6, 1.8, 2.7, 2.9 and 5.0 mg/kg.

The Meeting estimated a maximum residue level of 7 mg/kg. An STMR for the estimation of animal dietary burden was estimated to be 1.6 mg/kg.

Corresponding total residues of dicamba in rank order were: 0.83, 1.1, 1.1, 1.7, 1.7, 1.7, 1.9, 2.8, 2.9 and 5.1 mg/kg.

The Meeting estimated an STMR at 1.7 mg/kg.

The maximum residue level estimate derived from use of the NAFTA statistical calculator was 7 mg/kg, which was in agreement with the Meetings estimate.

Maize (field corn)

US GAP allows one to two applications per season, for a maximum seasonal application of 0.84 kg ai/ha. The normal use pattern consists of one application of 0.56 kg ai/ha applied pre-plant, pre-emergence or early post-emergence (up to the 5 leaf stage) and, if required, one application of 0.28 kg ai/ha applied late post-emergence (20–90 cm tall or 15 days before tassel emergence). No PHI was specified.

A total of 19 supervised field residue trials were conducted. The dimethylamine salt (DMA^+) of dicamba was applied in 11 trial locations. Side by side trials with three formulations (the dimethylamine salt (DMA^+), the diglycolamine salt (DGA^+), and the sodium salt (Na^+) of dicamba) were conducted at eight locations resulting in no significant effect of salt type on residues.

There was a fallow application in the previous fall. Since in the USA one season for maize is specified to be from March to October, this application is not regarded to be included in the maximum seasonal rate

Residues of dicamba from trials matching GAP were all < 0.01 mg/kg.

The Meeting estimated a maximum residue level of 0.01(*) mg/kg for maize. An STMR for the estimation of animal dietary burden was estimated to be 0.01mg/kg.

Corresponding total residues of dicamba in rank order were: < 0.02 (16), 0.02, (2) and 0.03 mg/kg.

The Meeting estimated an STMR at 0.02 mg/kg.

Sorghum

US GAP allows two applications: one application of 0.28 kg ai/ha immediately prior to the first joint stage and one application of 0.28 kg ai/ha applied at the soft dough stage. The PHI is 30 days.

A total of 11 supervised field residue trials were conducted.

Residues of dicamba from trials matching GAP were: 0.39, 0.41, 0.78, 0.97, 1.0, 1.2, 1.2, 1.3 and 2.0 mg/kg.

The Meeting estimated a maximum residue level of 4 mg/kg. An STMR for the estimation of animal dietary burden was estimated to be 1.0 mg/kg.

Corresponding total residues of dicamba in rank order were: 0.54, 0.85, 1.3, 1.7, 2.0, 2.2, 2.4, 2.7 and 3.2 mg/kg.

The Meeting estimated an STMR at 2.0 mg/kg.

The maximum residue level estimate derived from use of the NAFTA calculator was 3.5 mg/kg, which was in agreement with the Meetings estimate (after rounding up to one figure).

Wheat

US GAP allows two applications: one spring application of 0.28 kg ai/ha immediately prior to the first joint stage and one broadcast application of 0.28 kg ai/ha. The PHI is 7 days.

A total of 20 supervised field residue trials were conducted,

Residues of dicamba from trials matching GAP were: 0.05, 0.07, 0.08, 0.11, 0.11, 0.11, 0.16, 0.19, 0.19, 0.25, 0.29, 0.34, 0.35, 0.47, 0.53, 0.81, 0.84 and 1.1 mg/kg.

The Meeting estimated a maximum residue level of 2 mg/kg. An STMR for the estimation of animal dietary burden was estimated to be 0.22 mg/kg.

Corresponding total residues of dicamba were: 0.06, 0.09, 0.09, 0.12, 0.12, 0.16, 0.17, 0.20, 0.22, 0.30, 0.35, 0.37, 0.39, 0.50, 0.63, 1.1, 1.2 and 1.3 mg/kg.

The Meeting estimated an STMR at 0.26 mg/kg.

The maximum residue level estimate derived from use of the NAFTA calculator was 2 mg/kg, which was in agreement with the Meetings estimate.

Sugar cane

US GAP allows one application at 2.24 kg as/ha (2.24 kg as/ha total maximum seasonal application) applied at lay-by. Under these conditions, a PHI is not necessary.

A total of eight supervised field residue trials were conducted.

Residues of dicamba from trials matching GAP were: < 0.01, 0.01, 0.02, 0.03, 0.03, 0.05, 0.05, 0.96 mg/kg.

The Meeting estimated a maximum residue level of 1 mg/kg. An STMR for the estimation of STMR-P was estimated to be 0.03 mg/kg.

Corresponding total residues of dicamba were: 0.02, 0.03, 0.05, 0.08, 0.11, 0.13, 0.20 and 1.1 mg/kg.

The Meeting estimated an STMR and HR at 0.095 mg/kg and 1.1 mg/kg

The maximum residue level estimate derived from use of the NAFTA calculator was 1.3 mg/kg.

Cotton

The GAP of the USA allows a single pre-plant application at 0.28 kg as/ha. Residue trials were conducted at 0.56 kg ai/ha, which was the originally proposed application rate. Under this use condition, a PHI is not necessary.

A total of 12 supervised field residue trials were conducted. The LOQ was 0.04 mg/kg due to interference.

With the double rate, residues of dicamba were all < 0.04 mg/kg.

The Meeting therefore decided to estimate a maximum residue level of 0.04 (*) mg/kg for cotton seed.

As the LOQ is higher than those in other trials and application was made pre-plant possibly leading to nil residue situation, when both dicamba and 5-OH dicamba were < 0.04 mg/kg, the total residues were calculated to be < 0.04 mg/kg. When either dicamba or 5-OH dicamba was < 0.04 mg/kg and the other was higher than 0.04 mg/kg, residues at < 0.04 mg/kg were assumed to be zero in calculating the total residues.

Corresponding total residues of dicamba were: < 0.04 (11) and 0.05 mg/kg.

Since the trials were conducted at double rate with relatively high LOQ, the Meeting estimated both an STMR at 0.04 mg/kg.

Soya bean forage and hay

Soya bean forage and hay samples were collected before the second application is made to avoid abscission. Therefore, residues in these commodities came from pre-plant application.

Since the residues from the pre-plant application were expected to be very low and harvesting soya bean plants before harvesting soya bean seeds does not seem to be a common practice, the Meeting did not estimate a maximum residue level for soya bean forage and hay.

Barley and wheat straw

Since they are not distinguishable in trade, their trial results were evaluated together. US GAP for barley and wheat were similar.

Residues of dicamba in barley straw were: 1.0, 2.5, 3.1, 3.6, 3.6, 3.7, 5.5, 6.6, 10 and 30 mg/kg.

Residues of dicamba in wheat straw were: 0.40, 0.60, 1.1, 1.4, 2.2, 2.4, 2.4, 3.2, 3.6, 4.0, 4.4, 5.2, 5.3, 5.7, 7.1, 7.3, 21 and 23 mg/kg.

The Meeting concluded that the residue populations are similar and estimated a maximum residue level of 50 mg/kg. The highest dicamba residue and median residue for the estimation of animal dietary burden were 30 and 3.65 mg/kg for barley straw and 23 and 3.8 mg/kg for wheat straw.

The maximum residue level estimate derived from use of the NAFTA statistical calculator was 25 mg/kg based on barley straw data and 40 mg/kg based on wheat straw data.

Grasses forage and hay

The GAP of the USA allows one application of dicamba at 0.56 kg ai/ha. PHI for hay is 37 days and the shortest PHI for forage is 7 days.

The Meeting received trials data for various kinds of grasses, which are reviewed together in this evaluation.

Residues of dicamba in hay from those trials conducted according to GAP were: 1.4, 2.9, 3.1, 3.2, 3.4, 4.0, 6.3, 6.8, 6.9, 8.3, 8.6, 16 and 19 mg/kg.

The Meeting estimated a maximum residue level of 30 mg/kg for hay. The highest dicamba residue and median residue for the estimation of animal dietary burden were 19 and 6.3 mg/kg.

T The maximum residue level estimate derived from use of the NAFTA statistical calculator was 30 mg/kg, which was in agreement with the estimate of the Meeting.

Residues of dicamba in forage from those trials conducted according to GAP were: 2.2, 2.4, 6.6, 6.6, 6.9, 9.8, 11, 11, 12, 15, 15, 25 and 35 mg/kg.

The Meeting estimated the highest residue of 35 mg/kg and median residue of 11 mg/kg (fresh weight basis) for the calculation of animal dietary burden. These are equivalent to 140 mg/kg and 44 mg/kg on a dry weight basis after applying the dry matter of 25%.

Maize forage and fodder

Residues of dicamba in maize fodder from trials according to GAP were: 0.01, 0.01, 0.03, 0.04, 0.05, 0.06, 0.06, 0.06, 0.06, 0.06, 0.08, 0.08, 0.10, 0.10, 0.13, 0.24, 0.18, 0.20 and 0.33 mg/kg.

For trials on sweet corn, dicamba was applied twice instead of once as specified in GAP. However, even with two applications, residues of dicamba were mostly < 0.02 mg/kg and the highest residue was 0.05 mg/kg.

Based on trials on maize, the Meeting estimated a maximum residue level of 0.6 mg/kg for maize fodder. The highest dicamba residue and median residue for the estimation of animal burden were 0.33 and 0.06 mg/kg, respectively using the dry matter of 40%.

The maximum residue level estimate derived from use of the NAFTA statistical calculator was 0.60 mg/kg, which was in agreement with the estimate of the Meeting.

Residues of dicamba in forage from trials matching GAP were: 0.02, 0.06, 0.07, 0.07, 0.08, 0.09, 0.10, 0.12, 0.14, 0.16, 0.16, 0.18, 0.18, 0.19, 0.20, 0.25, 0.30, 0.30 and 0.31 mg/kg.

The Meeting estimated the highest residue and median residue for calculating animal dietary burden to be 0.31 and 0.16 mg/kg for maize forage on a fresh weight basis which are equivalent to 0.775 mg/kg and 0.40 mg/kg respectively.

Sorghum fodder

Residues of dicamba in sorghum fodder were: 0.57, 0.64, 0.81, 1.3, 1.3, 1.4, 1.6, 4.3 and 5.4 mg/kg.

The Meeting estimated a maximum residue level of 8 mg/kg. The highest dicamba residue and median residue for the estimation of animal dietary burden were 5.4 and 1.3 mg/kg.

The maximum residue level estimate derived from use of the NAFTA statistical calculator was 9 mg/kg.

Cotton gin trash

Residues of dicamba were even at double rate mostly < 0.04 mg/kg. In two trials residues of 0.05 and 0.06 mg/kg were observed. Since cotton gin trash is not an important trade item, no maximum residue level was recommended. The highest dicamba residue and median residue were 0.06 and 0.04 mg/kg. Although the trials were not in compliance with the current US GAP, the above mentioned values can be used for calculation of animal burden as an STMR and highest residue.

Fate of residues during processing

The Meeting received information on processing of soya beans to meal and oil; maize to flour, grits, meal, starch and oil; sugar cane to molasses and sugar; and cotton to meal and oil.

Processing factors were calculated for the processed commodities of the above and STMR-Ps for these commodities are shown below:

Processed Orange Product	Processing factor		STMR/STMR-P (mg/kg)
	Dicamba	Total residues	
Soya bean			
Refined oil	< 0.019	< 0.036	
Maize			0.02
Flour	0.26	0.28	0.0056
Large grits	0.20	0.22	0.0044
Meal	0.069	0.095	0.0019
Crude oil	< 0.029	< 0.058	0.00116
Wheat			0.26
Bran	0.99	1.0	0.26
Flour	0.052	< 0.070	0.02
Sugar cane			0.14
Molasses	42	24	3.4
White sugar	< 0.77	< 0.37	0.05
Cotton seed			0.04
Refined oil	< 0.01	< 0.02	0.008

As there is no concentration of dicamba and 5-OH dicamba observed in these processed commodities, no maximum residue levels are necessary for these commodities.

For the purpose of calculating animal dietary burden for estimating maximum residue levels for commodities of animal origin, STMR-P for maize hull and gluten, wheat bran and grain dust, sugar cane molasses and bagasse, and cotton seed meal were calculated based on dicamba residues only to be 0.0033, 0.014, 0.26, 2.3, 4.0, 0.198 and 0.07 mg/kg respectively..

Residues in Animal Products

Estimation of dietary burdens

Barley, maize, sorghum and wheat grains; soya bean forage and hay; straw of barley and wheat; grass forage and hay; processed maize byproducts; processed wheat byproducts; processed sugar cane products: and cotton gin trash and processed cotton byproducts may be fed to dairy cattle, beef cattle, broilers and layers. The maximum and dietary burdens were calculated using the highest residue or STMR/STMR-Ps of dicamba in commodities for which maximum residue levels were recommended on a basis of the OECD Animal Feeding Table.

5-OH Dicamba was not included in the calculation of animal burden as the feeding study with 5-OH dicamba resulted in very low uptake of 5-OH (< 0.01 mg/kg) into tissues, milk or blood of cattle at a dose equivalent to 59 ppm in the diet.

Summary of livestock dietary burdens (ppm of dry matter diet)

	US/CAN		EU		Australia		Japan	
	max	mean	max	mean	max	mean	max	mean
Beef cattle	6.0	2.6	71.2	23.2	140[a]	44.0[b]	15.7	5.8
Dairy cattle	64.3	21.1	85.0	27.4	140[c]	44.0[d]	27.5	9.2
Broilers	1.4	1.4	1.3	1.3	1.0	1.0	0.84	0.84
Layers	1.4	1.4	15.6[e]	6.0[f]	1.0	1.0	0.73	0.73

[a c] Suitable for estimating maximum residue levels for milk, meat, fat and edible offal of cattle.

[b d] Suitable for estimating STMRs for milk, meat, fat and edible offal of cattle.

[e] Suitable for estimating maximum residue levels for meat, fat and edible offal of poultry and eggs.

[f] Suitable for estimating STMRs for meat, fat and edible offal of poultry and eggs.

Residues in milk and cattle tissues

Lactating dairy cows were dosed daily for 30 consecutive days via gelatin capsules containing dicamba (40–400 ppm in diet).

Significant residues of dicamba and DCSA were found in kidney in all dose groups. However, in muscle, residues of dicamba and DCSA were generally low and < 0.01 mg/kg in the 40 ppm dose group. Residues in all tissues declined after withdrawal period to < 0.01 mg/kg in the 40 and 120 ppm dose groups. However, in kidney residues of 0.056 mg/kg was found in the 120 ppm group. Tissues from the 400 dose group contained significant amount of residues (0.01–0.28 mg/kg).

The maximum residues in milk were 0.023–0.039 mg/g in the 40 ppm dose group, 0.041–0.069 mg/kg in the 120 ppm dose group, and 0.21–0.34 mg/kg in the 400 ppm dose group. Residues in milk declined to < 0.01 mg/kg one day after termination of feeding dicamba.

In another study with 1000 ppm dose for 31 days, significant levels of residues were observed in liver (2.4–5.1 mg/kg) and kidney (9.8–47 mg/kg) . However, after 5 day withdrawal period, residues were reduced to 0.03 mg/kg in kidney and 0.22 mg/kg in liver. Muscle contained 0.11–0.39 mg/kg after 31 days of feeding period.

Milk contained up to 0.51 mg/kg of residues but within two days of withdrawal the concentration declined to < 0.01 mg/kg.

In a third study with 19, 59 and 183 ppm dose groups, no tissues contained residues above 0.01 mg/kg with an exception of 0.02–0.04 mg/kg occasionally found in kidney.

In the 183 ppm dose group, kidney contained up to 0.54 mg/kg of residues. In other tissues, residues above 0.01 mg/kg (up to 0.04 mg/kg) were found.

5-OH dicamba was fed to lactating cows at a dose rate equivalent to 19 ppm in the diet. However, the incorporation of 5-OH dicamba was minimal showing residues mostly below 0.005 mg/kg with occasional observation of 0.005 mg/kg.

Using the dietary burdens for beef and dairy cattle and the results in the lactating cattle feeding study, the maximum residue levels and STMRs were estimated. The calculated residues in cattle tissues and milk are summarized below.

Dietary burden mg/kg Feeding level [mg/kg]	Dicamba and DCSA residues, mg/kg				
	Milk	Muscle	Liver	Kidney	Fat
MRL	highest	highest	highest	highest	highest
140.0	0.071	0.016	0.082	0.331	0.036
[120/400]	[0.06/0.31]	[0.014/0.037]	[0.072/ 0.207]	[0.288/ 0.885]	[0.034/0.059]
STMR	mean	mean	mean	mean	mean
44.0	0.021	0.010	0.028	0.160	0.023
[40/120]	[0.02/0.04]	[< 0.01/0.012]	[0.026/0.066]	[0.154/0.282]	[0.023/0.025]

The Meeting estimated a maximum residue level for dicamba and DCSA in milks, mammalian meat, liver, kidney and fat at 0.2, 0.03, 0.2, 0.7 and 0.07 mg/kg. Based on the maximum residue levels for liver and kidney, the Meeting agreed to recommend a maximum residue level of 0.7 mg/kg for edible offal (mammalian).

STMRs were estimated to be 0.021, 0.010, 0.028 0.160 and 0.023 mg/kg for milks, mammalian meat, liver, kidney and fat. HRs were estimated to be 0.016, 0.082, 0.331 and 0.036 mg/kg for mammalian meat, liver, kidney and fat.

Residues in eggs and poultry tissues

Laying hens were fed with dicamba at a rate equivalent to 2, 6 and 20 ppm in the diet for 28 consecutive days. Tissues from the 2 and 6 ppm group, no residues above 0.01 mg/kg were observed except in liver up to 0.023 mg/kg of residues were found. In the 20 ppm dose group, up to 0.068 mg/kg of residues were found in liver, fat, skin and breast muscle. The residue concentration was lower in muscle than in other tissues.

Residues in pooled egg samples were < 0.01 mg/kg.

Using the dietary burdens for poultry broiler and layer and the results in the laying hen feeding study, the maximum residue levels and STMRs were estimated. The calculated residues in cattle tissues and milk are summarized below.

Dietary burden mg/kg Feeding level [mg/kg]	Dicamba residues, mg/kg			
	Eggs	Muscle	Liver	Fat
MRL	highest	highest	highest	highest
15.6	< 0.01	0.012	0.044	0.020
[6/20]	[< 0.01/< 0.01]	[< 0.01/0.013]	[0.023/0.053]	[< 0.01/0.025]
STMR	Mean	Mean	Mean	mean

Dietary burden mg/kg Feeding level [mg/kg]	Dicamba residues, mg/kg			
	Eggs	Muscle	Liver	Fat
6	< 0.01	< 0.01	< 0.01	< 0.01
[6]	[< 0.01]	[< 0.01]	[< 0.01]	[< 0.01]

The Meeting estimated a maximum residue level for dicamba and DCSA in eggs, poultry meat, liver and fat at 0.01*, 0.02, 0.07 and 0.04 mg/kg. Based on the maximum residue level for liver, the Meeting recommended a maximum residue level of 0.07 mg/kg for edible offal of poultry.

STMRs were estimated to be 0.01, 0.01, 0.01 and 0.01 mg/kg for eggs, poultry meat, liver and fat. HRs were estimated to be 0.01, 0.012, 0.044 and 0.01 mg/kg for these commodities.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Dietary Intakes (IEDIs) of dicamba were calculated for the 13 GEMS/Food cluster diets using STMRs and STMR-Ps estimated by the current Meeting (Annex 3). The ADI is 0–0.3 mg/kg bw and the calculated IEDIs were 0–1% of the maximum ADI. The Meeting concluded that the long-term intake of residues of dicamba resulting from the uses considered by the current JMPR is unlikely to present a public health concern.

Short-term intake

The International Estimated Short-Term Intakes (IESTI) of dicamba were calculated for food commodities and their processed commodities using HRs/HR-Ps or STMRs/STMR-Ps estimated by the current Meeting (Annex 4). The ARfD is 0.5 mg/kg and the calculated IESTIs were 0–4% of the ARfD for the general population and 0–9% of the ARfD for children. The Meeting concluded that the short-term intake of residues of dicamba, when used in ways that have been considered by the JMPR, is unlikely to present a public health concern.