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DATA EVALUATION RECORD

STUDY TYPE: Metabolism - rat
[OPPTS 870.7485 [§85-1)]

DP BARCODE: D243609 SUBMISSION CODE: S538189
P.C. CODE: 108800 TOX. CHEM. NO.:

TEST MATERIAL: 2-Chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-acetamide: (S)-enantiomer and racemic mixture both radiolabelled in the phenyl [U-^{14}C] ring.

SYNONYMS: CGA-77102 (or Metolachlor-S), and CGA-24705 (or Metolachlor)

CITATIONS : Muller, T. 1996. "Comparison of Metabolite Pattern in Rat Excreta after Administration of [Phenyl-U-^{14}C] CGA-77102 and [Phenyl-U-^{14}C] CGA-24705". Animal Metabolism, Product Safety Division, Novartis Crop Protection AG, Basle, Switzerland: PR 18/96 (Novartis No. 490-96) November 22, 1996. Laboratory Project: 30AM02. MRID No.: 44491402. Unpublished

SPONSOR: Novartis Crop Protection, Inc., P.O.Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY:

The rat urinary and fecal metabolite patterns were compared following the administration of [Phenyl-U-^{14}C] CGA-77102 (S-Metolachlor) (Groups B1, D1, V1, G1, and G2) from the accompanying study (MRID 44491401) and after administration (3 rats/sex) of a single oral low dose (0.5 mg/kg, Group B2) of [Phenyl-U-^{14}C] CGA-24705 (R/S-Metolachlor, racemate). The pooled urine and feces (0-48 or 0-72 hours) from each group were subjected to clean-up or extraction prior to further analysis by 2-dimensional thin layer chromatography (2-D TLC). Bile fluid (genuine) from Groups G1 and G2 was also analyzed by 2D TLC. Selected bile and urine specimens were also examined for metabolite stability during the storage period lasting up to five months. Biliary metabolites seemed to be stable under the storage conditions. However, two out of three tested urine specimens showed large metabolite profile changes in at least eight of the 18 total fractions before and after storage. The study report did not explain how these results might impact the interpretation of the comparative metabolite profiles.

The 72 hour mean recovery of radioactivity in urine, feces, and carcass following administration of 0.5 mg/kg of [Phenyl-U-^{14}C] CGA-24705 was 43.1%, 47.0%, and 7.4% in

males and 54.0%, 39.4%, and 4.1% in females, respectively. In contrast, both sexes excreted more of the label in the feces (M:F 59.7%:53.4%) than in the urine (M:F 29.4%:39.8%) during the same period following administration of the same dose of [Phenyl-U-^{14}C] CGA-77102 (the S-enantiomer) (MRID 44491401).

The urinary and fecal metabolite profiles, with 31 and 15 metabolite fractions, respectively, were qualitatively similar among all groups; however, there were large quantitative differences, based on the dosing formulation, on one hand, and the sex of the animal, on the other. Based on a percentage of the dose, several of the major urinary metabolite fractions (e.g., U1, U2, U3, U18, U24, and U30) were more abundant in the case of the racemic-Metolachlor (CGA-24705) than the S-Metolachlor (CGA-77102); in contrast, several fecal metabolite fractions (e.g., F9, F10, F12, and F13) were present at higher levels in the case of CGA-77102 than CGA-24705. On the other hand, there were sex-related differences regardless of the dosing formulation where, for instance, females had greater urinary concentrations than males of several metabolite fractions, including U3, U4, U8, U9, U18, U20, and U30; the males, however, excreted more of fractions U1 and U24 than the females. Also, several fecal fractions including F1, F3, F5, F6, F7, F8, and F13 were influenced by the sex regardless of the dose level (e.g. B1 vs. D1) or the stereochemical make-up of Metolachlor (B1 vs. B2). Other metabolite fractions were dependent on both the sex and the chemical formulation as, for instance, in the case of metabolite U2 which, relative to the opposite sex within the same group, was more abundant in the urine of the females of Group B2 (CGA-24705) and in the urine of the males of Group B1 (CGA-77102).

The bile fluid accounted for 79.8% of the administered low or high dose of CGA-77102 (Groups G1 and G2) where the 2D-TLC showed 14 biliary metabolite fractions (G1-G14) in the high dose Group and only six metabolites in the low dose Group. The two metabolite fractions G7 and G8 accounted, respectively, for 33.3% and 9.6% of the administered low dose and 31.3% and 14.6% of the administered high dose. Other major biliary metabolites were G3, G9, and G10 which accounted for about 5%, 5-7%, and 3-5%, respectively, of either dose group.

The results clearly show that the metabolite profile in excreta and bile fluid is very complex and that Metolachlor (racemate or S-enantiomer) is extensively metabolized. This was also shown earlier by another rat metabolism study on the absorption, distribution, excretion, and metabolite identification of racemic CGA-24705 (MRID 43164201, reviewed by T. McMahon, HED doc. no. 010990 dated May 23, 1994). No actual metabolites or pathways were identified in the current study and there were no data to support or refute the previous findings of four major degradation pathways with more than 30 metabolites. However, knowing the enantiomeric stereospecific reactions/metabolites is not likely to help in making comparative risk assessments between R/S-Metolachlor (CGA-24705) and S-Metolachlor (CGA-77102) since the contribution of each metabolite to the overall toxicity of Metolachlor is not well understood. Furthermore, other bridging animal studies with CGA-77102 should highlight possible toxicity differences from the well-studied CGA-24705 due to variations in the metabolite profiles.

The Registrant is requested to comment on or provide information on a number of issues

including: 1) The stereoisomeric purity of CGA-24705 and CGA-77102. 2) The adequacy of the storage conditions and the validity of the metabolite profile results in light of the storage-related results variability. 3) Explain why, relative to the other dosing formulation, some metabolite fractions (e.g., F10, F12, and F13) were up to 7-fold higher in the case of the S-enantiomer (CGA-77102) while some urinary metabolite fractions (e.g., U1, U2, and U3) were up to 4-fold higher in the case of CGA-24705. 4) Provide rational for dose selection. 5) The Registrant might also have to comment on the possible formation and the level of methylethylaniline from either dosing formulation and the possible contribution of this metabolite to the carcinogenicity of Metolachlor. This issue was raised earlier by T. McMahon (HED document no. 010990 dated May 23, 1994) and might need to be followed up by HED's risk assessors who are in charge of S-Metolachlor.

CLASSIFICATION. This study is classified as **Guideline/UNACCEPTABLE**. However, this study might be Upgradable, pending the receipt of the Registrant's responses to the requested information and further assessment by HED.

COMPLIANCE: Signed and dated statements were provided for GLP (Switzerland, March 1986 based on the OECD GLP of May 12, 1981), Quality Assurance, and Data Confidentiality.

I. MATERIALS AND METHODS AND STUDY DESIGN

A. MATERIALS:

1. Test Compound:

Radiolabeled: [Phenyl-U-^{14}C] CGA-77102; Sp. Activity: 51.35 µCi/mg (1.90MBq/mg)
Batch: GAN-XXXV-59-1; Purity: >98 %
[Phenyl-U-^{14}C] CGA-24705; Sp. Activity: 40.5 µCi/mg (1.50MBq/mg)
Batch: ILS-94.1; Purity >99 %

Non-radioalabeled: CGA-77102; CAS Reg. No. 87392-12-9
Batch: AMS 757/101; Purity: >99 %
CGA-24705; CAS Reg. No. 51218-45-2
Batch: AMS 124/108; Purity: >99 %

Optical Purity: Not specified for the S-enantiomer CGA-77102
Assumed to be 50:50 of the R- and S-enantiomers in CGA-24705

Description: Clear liquid.
Contaminants: No information.

2. Vehicle: Ethanol/polyethylene glycol 200/water 3/2/3 (v/v)

3. Test animals:

Species: Rat
Strain: Tif: RAI f (SPF)
Age: 7 - 9 weeks
Weight at study initiation: 200-250 g
Source: Ciba-Geigy, Switzerland, Animal Production, Stein
Housing: Individually one day before dosing with the labeled material
Diet: Certified standard diet (Nafag No. 890), ad libitum
Water: Tap water (ad libitum) for all groups except for groups G1 and G2 which, following the bile cannulation surgery, were given water containing 5% glucose, 0.9% NaCl, and 0.05%KCl.

B. STUDY DESIGN: The design of the study using (S)-Metolachlor (CGA-77102) was specified in the accompanying Data Evaluation Record for MRID 44491401. Urine and fecal specimens of Groups B1, V1, D1, G1, and G2, in addition to bile from Groups G1 and G2, were used for analysis in the present study. An additional group (B2) of three animals of either sex was administered an oral nominal dose of 0.5 mg/kg of CGA-24705 (racemic Metolachlor) and

the excreta were used for comparative analysis with the samples from the rats given S-Metolachlor (CGA-77102). The following is a summary of the study design and the pools that were analyzed for metabolite profiling.

TABLE 1: Study Design and Pools Used for Metabolic Profiling*

Group	Test Substance	Dose	Rats/sex	Type of Specimen	Sampling Time (hr)	Amount (% of Dose)	Designation of Pool
B1	CGA-77102	~0.5 mg/kg (5.2 µCi)	5 Males	urine	0 - 48	27.2	U1/B1m
				feces	0 - 72	59.6	F1/B1m
			5 Females	urine	0 - 48	36.6	U1/B1f
				feces	0 - 72	53.3	F1/B1f
V1**		~0.5 mg/kg (5.2 µCi)	5 Males	urine	0 - 48	33.2	U1/V1m
				feces	0 - 72	57.9	F1/V1m
			5 Females	urine	0 - 48	41.7	U1/V1f
				feces	0 - 72	47.1	F1/V1f
D1		~100 mg/kg (203.3 µCi)	5 Males	urine	0 - 72	33.1	U1/D1m
				feces	0 - 72	57.5	F1/D1m
			5 Females	urine	0 - 72	42.5	U1/D1f
				feces	0 - 72	45.7	F1/D1f
G1		~0.5 mg/kg (6.3 µCi)	6 Males	urine	0 - 48	4.6	U1/G1m
				feces	0 - 48	2.4	F1/G1m
				bile fluid	0 - 48	79.8	G1/G1m
G2		~100 mg/kg (198.3 µCi)	6 Males	urine	0 - 48	3.0	U1/G2m
				feces	0 - 48	2.2	F1/G2m
				bile fluid	0 - 48	79.8	G1/G2m
B2	CGA-24705	~0.5 mg/kg (4.1 µCi)	3 Males	urine	0 - 72	43.1	U1/B2m
				feces	0 - 72	47.0	F1/B2m
			3 Females	urine	0 - 72	54.0	U1/B2f
				feces	0 - 72	39.4	F1/B2f

* Information in this Table are from Table of page 20 of the study report (MRID 44491402).
** Animals in group V1 were administered 14 daily oral doses of 100 mg/kg of non-radiolabeled CGA-77102.

C. METHODS:

The methods for the preparation and administration of the dosing solutions, specimen collection and storage, and radioactivity measurement were summarized in the accompanying DER on the toxicokinetics study with CGA-77102 (MRID 44491401). The following is a brief summary of the additional methods that were used in this study.

1. Preparation of Dosing Solutions and Administration: (Refer to page 19 of study report)

The dosing solutions were prepared by dissolving the radiolabeled test substance in the vehicle (above) so that each animal received (by stomach tube) about 0.8 ml of the dosing

formulation.

According to the report, the test substance in the administration solution was analyzed by TLC at the time of dosing and was found to be stable with CGA-24705 representing > 98% of the radioactivity. Two representative TLC radiochromatograms of the administration solution under normal and reversed phase conditions were provided (Figure 2 of report).

2. Specimen Collection and Storage:

Urine and feces samples were individually and separately collected on dry ice and at room temperature, respectively. The urine and feces specimens from CGA-24705 treated rats were collected at the post-dosing intervals of 0 - 24, 24 - 48, and 48 - 72 hours. Volumes and weights of all specimens were recorded prior to analysis. The animals were killed (with an overdose of CO_2) three days after dosing and the carcass was kept for analysis. Until analyzed, urine, feces, and the carcass were kept at -18°C. The specimen pools that were used for metabolite profiling are listed in Table 1 above.

3. Storage Stability: (Refer to page 21 of the study report)

The storage stability (during nearly four to seven months) was examined by comparing the quantitative metabolite pattern of selected specimens from rats treated with CGA-77102 after collection and at the end of the analytical period. The excreta from Group B2 (CGA-24705 treated) were not examined for storage stability because the analysis was completed within 30 days of specimens collection.

4. Radioactivity Measurement: (Refer to pages 21-22 of the study report)

Radioactivity was measured by liquid scintillation counting (LSC) using a Packard Tri-Carb Counter. Measured aliquots of all liquid specimens, including feces extract, were added directly to the scintillation cocktail. After adding water, feces were manually homogenized with a pestle; the carcasses were homogenized in dry ice using a food chopper. Specimens of feces, non-extractable fecal solids, or carcass were combusted in a special sample oxidizer prior to counting the trapped CO_2. Based on appropriate tests of the sample oxidizer, recoveries were above 95% and carry-over below 0.5%.

The metabolite pattern in excreta was examined following chromatography by TLC (below) and visualization by an image analyzer with capabilities for scanning and electronic integration of the radioactive fractions as described in more detail on page 22 of the study report.

5. Thin Layer Chromatography (TLC): (Refer to pages 22-23 of the study report)

Analyses were performed using two-dimensional silica gel TLC (normal phase) with the appropriate solvent systems for metabolites resolution from urine, feces, or bile. The stability of

CGA-24705 in the administration solution was assessed by reversed-phase RP-18 TLC. Additional details are given on pages 22 and 23 of the study report.

6. Preparation of Specimens and Other Methods:

The urine pools from the low dose groups were cleaned-up and enriched using solid-phase extraction (RP-18 cartridges) as outlined in Figures 3 - 6 of the study report which are included as an attachment with this DER.

The feces pools (in acetonitrile) were column chromatographed (type not specified) and eluted with acetonitrile/water (4/1) to yield **extract 1**. The non-extractable solids were subjected to an exhaustive 16 hour Soxhlet extraction step to yield **extract 2** leaving behind a non-extractable **residue**. The extractibility results of the radioactivity in feces (Table 5 of the study report) are included as an attachment with this DER. **Extract 1** was further analyzed by 2-dimensional TLC.

Bile fluids were analyzed directly by 2-dimensional TLC.

7. Calculations and Statistics:

No statistical tests were used to assess the results and this is considered by RAB-III to be appropriate. Mean values and recoveries were calculated as shown on page 24 of the study report (also included as an attachment with this DER).

II. RESULTS

A. Storage Stability of Specimens

The metabolite patterns of urine and bile were quantitatively compared at the beginning and at the end of the storage periods (Tables 8, 9 and Figure 10 of MRID 44491402). The results of the storage stability of two bile specimens did not show major variability in any of the twelve fractions when analyzed soon after collection and four months later (Table 9 of the study report). The urinary metabolite pattern of pool SU1/V1f (from animal no. 95436) exhibited marked quantitative changes where, following more than five months of storage, each of the polar Fr 1, Fr 2, and Fr 4 were decreased while the non-polar Fr 17 and Fr 18 were increased with the total net decrease in the polar fractions(from 30.9% before storage to 8.3% after storage) being equal to the net increase (from 3.2% before storage to 25.8% after storage) in the non-polar fractions (copy of Table 8 included as an attachment with this DER). Other fractions, including Fr 12, Fr 15, and Fr 18 in pool SU1/B1f from animal no. 95423, seemed to also have different concentration before and after storage. On the other hand, relatively minor or no storage-related changes were observed in any of the fractions from the third urinary pool of SU1/D1f from animal no. 95412. It is not clear why some of the stored urine specimens but not others seem to

yield variable results which the study report did point out but did not explain or comment on, aside from concluding that the results "are considered not to be affected essentially by the length and conditions of storage of the specimens analyzed." In this reviewer's opinion, however, these results, in total, do cast some doubt about the adequacy of the storage conditions and about the validity of the metabolite fraction results.

B. Disposition of CGA-24705

Of the administered dose of 0.5 mg/kg of [Phenyl-U-^{14}C] CGA-24705, the 72 hour mean recovery of radioactivity in urine, feces, and carcass was 43.1%, 47.0%, and 7.4% in males and 54.0%, 39.4%, and 4.1% in females, respectively. In this study, the males excreted relatively more of the label in feces than in urine while females had a larger percentage of the label in their urine over the feces. In the accompanying study (MRID 44491401), both sexes excreted more in the feces (M:F 59.7%:53.4%) than in the urine (M:F 29.4%:39.0%) during the same period after [Phenyl-U-^{14}C] CGA-77102 (the S-enantiomer) was administered at the same dose of 0.5 mg/kg. It is not clear if these observed differences are due to the use of a racemic mixture (CGA-24705) vs. an enriched S-enantiomer (CGA-77102) or are simply due to random or chance experimental variations between the two studies. Nonetheless, these disposition results do not lend support to the hypothesis that Metolachlor has the same excretory fate whether it is administered as the S-enantiomer or the racemic mixture.

C. Urinary Metabolite Pattern

As explained above under "Methods: Preparation of Specimens," the urine pools from the low dose groups (B1, V1, G1, and B2) were cleaned-up and enriched using solid-phase extraction (RP-18 cartridges). In each case, the majority of the radioactivity was present in the methanolic eluate which was analyzed by 2-dimensional silica gel TLC under normal phase conditions where the fractions are separated according to their polarity, with the most polar being retained near the origin while the least polar are closer to the solvent front. Genuine urine from the high dose groups (D1 and G2) was analyzed directly by 2-dimensional TLC. Based on the 2-dimensional TLC profile, the quantitative distribution (expressed as % of dose) of 31 metabolites (U1-U31) was summarized in the Table of page 26 (copy attached with this DER). In Table 4 of study report, the same data was also expressed as % of radioactivity present in urine or clean-up fraction (not shown with this DER). It is not clear why, herein, 31 urinary metabolite fractions (U1-U31) were identified and quantitated while in the storage stability study (Figure 10 and Table 8), only 18 fractions (termed Fr 1-Fr 18) were evaluated (discussed above and copy of Table 8 included). This could be somewhat confusing; however, it might be best not to consider the two types of urinary metabolite fractions related and to focus, instead, on the 31 metabolites (U1-U31) for profiling.

Similar qualitative metabolic profile can be seen for all urine specimens, irrespective of the dose, sex, or the optical purity of the chemical, i.e. the racemic mixture (CGA-24705) vs. the S-enantiomer (CGA-77102) (see the attached table of page 26 of study report). However,

quantitative differences were seen in several instances. The most noticeable difference between the two chemical formulations is the nearly two to four fold higher level in each of the most polar fractions U1, U2, and U3 from males and females of the CGA-24705 treated group compared to the same sex animals that were administered CGA-77102. Another noticeable difference between the two chemical formulations is that the major fraction U18 from rats given CGA-24705 (% of total urinary metabolites from males: females = 8.9%:12.3%) is nearly 50 - 100% higher than the same major fraction from CGA-77102 (M:F = 4.3%:8.2%).

On the other hand, there were sex-related differences in the abundance of several urinary fractions from rats given either chemical formulation (attached table of page 26 of study report). For instance, in the case of CGA-77102, males of Groups B1, V1, and D1 excreted relatively more of U1 and U2 than the females which, in contrast, excreted more of U3 and U4 than did the males in the same groups. Likewise, the females of Group B2 (CGA-24705) eliminated more U3 and U4 in addition to U2, while U1 was relatively more abundant in the urine from the males. Other sex-related differences could be seen in the levels of U8, U9, U18, U20, U24, and U30 all of which (except U24) were more abundant in the urine of females than males, irrespective of the chemical stereospecificity of the dosing formulation. The abundance of these metabolites, weighted, in most instances, in favor of the females over the males, should explain the overall higher urinary excretion of radiolabel by females than males. On the other hand, the larger overall urinary excretion of radiolabel from CGA-24705 compared to CGA-77102, might be explained by the higher abundance of the CGA-24705 derived metabolites U1, U2, U3, U18, U24, and U30, in addition to R (unresolved radioactivity) compared to the same metabolites from CGA-77102.

In conclusion, the urinary metabolic profile, while qualitatively similar between all treated groups, seems to highlight some quantitative differences based on the sex and the stereochemical composition of the dosing formulation. Both sexes in Group B2, which was administered the racemic mixture (CGA-24705), excreted more of several metabolite fractions than did rats in any of the groups that were treated with the S-enantiomer (CGA-77102). This could mean that the R-enantiomer in CGA-24705 is also susceptible to metabolism and contributes to the abundance of certain urinary metabolite fractions.

Finally, none of the urinary metabolites was structurally characterized.

D. Fecal Metabolite Pattern

Of the total radioactivity present in feces from the CGA-77102 treated groups (B1, V1, and D1), nearly 78 - 86% were recovered in Extract 1; similar recovery (74 - 78%) was also found in the fecal Extract 1 from the CGA-24705 treated Group B2 (see attached Table 5 from the study report). The metabolic profile was examined by subjecting Extract 1 to 2-dimensional silica gel TLC under normal phase conditions where the most polar fractions are retained near the origin while the least polar are closer to the solvent front. Based on the 2-dimensional TLC profile from all groups, the quantitative distribution (expressed as % of dose) of 15 metabolites (F1-F15)

was summarized in the Table of page 28 (copy attached with this DER). In Table 6 of study report, the same data was also expressed as % of radioactivity present in fecal Extract 1 (not shown with this DER). Based on the attached Table of page 28, all groups had a similar qualitative profile; however, the dose, the sex, and the stereochemical formulation seemed to influence the relative abundance of most fractions. For instance, F12 seems to be affected by the dose level where in males/females of the low dose Group B1 and the high dose Group D1 it represents nearly 2.0/2.5% and 11.1/13.2%, respectively, of the administered dose. An opposite dose effect was seen with F13 which, relative to the administered dose, was higher in Group B1 (M/F: 3.9/7.5%) than in Group D1 (M/F: 1.0/1.4%). In females only, the relative abundance of F3, 6.3% and 2.1% of the administered low dose (B1) and high dose (D1), respectively, was also affected by the dose. On the other hand, F3 and other fractions including F1, F5, F6, F7, F8, and F13 were influenced by the sex of the animal, irrespective of the dose level (e.g. B1 vs. D1) or the stereochemical make-up of Metolachlor (B1 vs. B2). Other observations are outlined as follows:

- While the metabolite patterns were qualitatively similar between the groups administered CGA-77102 (B1) and CGA-24705 (B2), there were some quantitative differences, most notably in F9, F10, F12, and F13, all of which were more abundant in Group B1 compared to Group B2. These differences might, at least partly, account for the larger overall fecal excretion (as % of administered dose) by the first group. These quantitative differences suggest that the metabolism of Metolachlor is affected by its stereoisomeric composition.

- With minor exceptions (e.g., F4 and F13), the relative male/female abundance of most fractions was similar between the two groups given CGA-77102 (B1) and CGA-24705 (B2). The overall similar male/female metabolic pattern suggest that there is little or no stereoisomeric effect on Metolachlor metabolism.

- Unchanged CGA-77102 (F14) accounted for about 1-3% of the dose. The report did not specify if fraction F14 in Group B2 also represented unchanged CGA-24705.

- Among the principal metabolites, in at least one dose group and one sex, are F3, F5, F7, F9, F12, and F13.

Finally, none of the fecal metabolites was structurally characterized.

E. **Metabolite Pattern in Bile Fluid**

The bile fluid from the bile-duct cannulated male rats, which accounted for 79.8% of the administered low or high dose of CGA-77102 (Groups G1 and G2), was analyzed directly by silica gel 2-dimensional TLC under normal phase conditions. There were 14 metabolite fractions (G1-G14) in the high dose Group G2; however, only six biliary metabolites were seen in the low dose Group G1 (data not shown here but may be found in Table 7, and table of page 29 of the

study report). The two metabolite fractions G7 and G8 accounted, respectively, for 33.3% and 9.6% of the administered low dose and 31.3% and 14.6% of the administered high dose. Other major biliary metabolites were G3, G9, and G10 which accounted for about 5%, 5-7%, and 3-5%, respectively, of either dose group. Of the administered dose, the fraction of unresolved biliary radioactivity (termed R) was relatively higher in the low dose than the high dose group (21.2% and 8.5%, respectively).

Finally, none of the biliary metabolites was structurally characterized.

III. DISCUSSION

A. Conclusions of the Study Report

The author of the study report concluded that the disposition of CGA-24705 (R/S-racemate) corresponds to that of CGA-77102 (S-enantiomer) and, except for minor quantitative differences in some of the metabolite fractions, the same urinary or fecal metabolite pattern was seen in rats administered either formulation. Hence, the metabolic pathways of CGA-24705 should be valid for CGA-77102. Following administration of CGA-77102, the urinary and fecal metabolite patterns were independent of the dose level, pretreatment, or the sex of the animal; also, the biliary metabolite pattern was not influenced by the dose level.

CGA-77102 was extensively metabolized since the urine, bile, and feces had up to 31, 14, and 15 metabolite fractions, respectively; the major urinary (U18) and fecal (F12) metabolites did not exceed 8% and 13% of the dose, respectively, while G7, the major biliary metabolite, represented nearly one third of the dose. Since the metabolite pattern in feces differs from that in the bile fluid, it is evident that the biliary metabolites are partially reabsorbed to undergo further metabolism and elimination via bile and kidneys. The feces contained a small portion (1-3% of the dose) of unchanged CGA-77102 (metabolite F14), which, based on the presence of additional small metabolite fractions in the feces of bile-duct cannulated rats, is assumed to undergo partial degradation in the gastrointestinal tract.

B. Reviewer's Comments

The main objective of the current study was to compare the metabolic profiles of CGA-27405 (R/S-Metolachlor) against CGA-77102 (S-Metolachlor) following a single oral administration of either formulation to male and female rats at a nominal dose level of 0.5 mg/kg. Other objectives were to compare the influence on the metabolic profile of the sex, the dosage, and single vs. repeated exposure to Metolachlor. The results clearly show that the metabolite profile in excreta and bile fluid is very complex and that Metolachlor (racemate or S-enantiomer) is extensively metabolized. This was also shown earlier by another rat metabolism study on the absorption, distribution, excretion, and metabolite identification of racemic CGA-24705 [Phenyl-(U)-^{14}C]-Metolachlor (MRID 43164201, reviewed by T. McMahon, HED doc.

no. 010990 dated May 23, 1994).

The urinary and fecal metabolite profiles were qualitatively similar among all groups; however, as described under results, there were large quantitative differences, based on the stereochemical formulation, on one hand, and the sex of the animal, on the other. Based on a percentage of the dose, several of the major urinary metabolite fractions (e.g., U1, U2, U3, U18, U24, and U30) were more abundant in the case of the racemic-Metolachlor (CGA-24705) than the S-Metolachlor (CGA-77102); in contrast, several fecal metabolite fractions (e.g., F9, F10, F12, and F13) were present at higher levels following the administration of CGA-77102 than CGA-24705. In the absence of additional information, these differences can not easily be explained but do point out to possible stereospecific biotransformation reactions. It is possible that different enzyme-catalyzed reactions have preference or specificity to one or the other stereoisomer. In other words, an X metabolic reaction might be specific towards the R-enantiomer while the S-enantiomer is the preferable substrate for a different Y reaction. It is likely that one or more of the cytochrome P-450 catalyzed oxidation steps is stereospecific. This study, per se, was not designed to shed light on which of the two enantiomers is specifically metabolized by which metabolic pathways. Such studies could be very labor intensive and costly. Also, additional details on the stereospecificity of Metolachlor biotransformation could be more relevant from an academic than a regulatory perspective.

Furthermore, while two transformation schemes with more than 30 metabolites have been proposed for Metolachlor (see MRID 43164201 or HED document no. 010990 dated May 23, 1994), the contribution of each metabolite to the overall toxicity of Metolachlor is not well understood. Therefore, knowing the stereospecific reactions/metabolites pertaining to R- or S-Metolachlor is unlikely to be practically useful for making comparative safety assessment between R/S-Metolachlor (CGA-24705) and S-Metolachlor (CGA-77102). Furthermore, other bridging animal studies with CGA-77102 should highlight possible toxicity differences from the well-studied CGA-24705 due to variations in the metabolite profiles.

Other comments include the following:

- It is difficult to explain why specific fecal metabolite fractions (e.g., F10, F12, and F13) were 3 - 7 fold higher in the case of the S-enantiomer (CGA-77102) compared to the racemic formulation (CGA-24705) when the relative amount of the S-enantiomer (assuming 100% pure) only doubled relative to its 50% concentration in the racemate mixture. In other words, if these metabolites were preferentially generated from the S-enantiomer, why weren't they increased proportionately with the 2-fold increase in this enantiomer ? The Registrant is requested to comment.

- Since the fractionation schemes that were used in the previous study (HED document no. 010990 dated May 23, 1994) are different from the ones used in the present study, there is little or no correspondence between the designated urinary and fecal fractions of both studies. Up to 47 urinary and 42 fecal metabolite fractions were listed in the previous

Metolachlor study (HED document no. 010990 dated May 23, 1994), while 31, and 15, respectively, were listed here. Therefore, it is possible that one or more of the TLC metabolite fractions in this study might, each, be composed of more than one chemical. Without using additional analytical methods (e.g., MS or NMR) to verify purity, structurally related metabolites might not be adequately resolved by the 2-dimensional TLC methods used; therefore, a designated TLC spot (or fraction) can not automatically be interpreted to represent a single chemical species.

- The issue previously raised by T. McMahon (HED document no. 010990 dated May 23, 1994) on the formation of methylethylaniline and its possible contribution to the carcinogenicity of Metolachlor might also be relevant here. Therefore, the Registrant might have to comment on the possible formation and the level of methylethylaniline from S-Metolachlor (CGA-77102) in comparison to that from R/S-Metolachlor (CGA-24705). This issue is being raised here but the decision, as to whether the Registrant needs to provide this information, is deferred to the HED scientist(s) responsible for performing the risk assessment on S-Metolachlor.

- The Registrant did not justify the dose levels (0.5 and 100 mg/kg) selected for the pharmacokinetics (MRID 44491401) and metabolite pattern studies (MRID 44491402) of the S-enantiomer. Higher doses (1.5 and 300 mg/kg) were selected in the previous studies with the racemate mixture (CGA-24705). The Registrant is requested to comment.

- Finally, this reviewer concurs that this study is adequate for the intended purposes and that the metabolic pathways of CGA-24705 should be valid for CGA-77102. This is especially true since our understanding of the significance of the individual degradation steps or metabolites is limited, especially in terms of influencing the risk assessment process. Four major degradation pathways were previously outlined including, cleavage of the methyl ester, oxidation of the resultant alcohol, glutathione conjugation of the chloroacetyl group followed by hydrolysis, and oxidation of the aryl methyl and/or ethyl groups to benzylic alcohols followed, in some cases, by cyclization (HED doc. no. 010990 dated May 23, 1994). There were no actual data in this study to support or refute any of these pathways.

IV. CONCLUSIONS

The Registrant is requested to comment or provide information on the following issues that were raised earlier in this DER:

1) Specify the stereoisomeric purity of CGA-24705 and CGA-77102.

2) As discussed above (II-A), it is not clear why, following several months of storage, some of the urine specimens but not others seem to yield variable metabolite profiles that are suggestive of decomposition. Therefore, this reviewer questions the validity of the

CGA-24705 and CGA-77102 metabolite profile results even though the author of the study report discounted any effects due to the length and conditions of the specimen's storage. The Registrant needs to reassess the adequacy of the storage conditions and comment on the validity of the metabolite profile results in light of the storage-related variability.

3) Explain why some metabolite fractions (e.g., F10, F12, and F13) were 3 - 7 fold higher in the case of the S-enantiomer (CGA-77102) compared to the racemic formulation (CGA-24705) when the relative amount of the S-enantiomer (assuming 100% pure) was, at the most, twice its concentration in the racemate mixture. On the other hand, some urinary metabolite fractions (e.g., U1, U2, and U3) were nearly 2 - 4 fold higher following administration of CGA-24705 than CGA-77102. Does this mean that these urinary metabolites are preferentially generated from the R-enantiomer and how is this observation related to the stereoisomeric composition of the two Metolachlor preparations ?

4) Explain why different dose levels (0.5 and 100 mg/kg) were selected for the pharmacokinetics (MRID 44491401) and metabolite pattern studies (MRID 44491402) of the S-enantiomer while higher doses (1.5 and 300 mg/kg) were used in the previous studies with the racemate mixture (CGA-24705).

5) The scientist(s) within HED who are responsible for performing the risk assessment on S-Metolachlor might need to follow up on the issue raised by T. McMahon (HED document no. 010990 dated May 23, 1994) regarding the formation of methylethylaniline and its possible contribution to the carcinogenicity of Metolachlor. The Registrant might have to comment on the possible formation and the level of methylethylaniline from S-Metolachlor (CGA-77102) in comparison to that from R/S-Metolachlor (CGA-24705).

This study is classified as **Guideline/UNACCEPTABLE**. However, this study might be Upgradable, pending the receipt of the Registrant's responses to the requested information and further assessment by HED.

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Reference to these attachments in the text states the purpose for including these attachments.

All attachments are from MRID No.: 44491402.

List of Attachments

Figure 3 - 6: Clean-up of urine from Groups B1, V1, G1, and G2

Table 5: Extractability of radioactivity from feces of male and female rats after oral administration of [Phenyl-U-^{14}C] CGA-77102 and [Phenyl-U-^{14}C] CGA-24705

Table 8: Storage stability of urine

Page 24: Calculations

Page 26: Quantitative metabolite pattern in urine [% of dose]

Page 28: Quantitative metabolite pattern in feces [% of dose]

MRID 44914 02

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