UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

013221

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OFFICE OF
THE ADMINISTRATOR

MEMORANDUM

SUBJECT: Acetochlor - Tox. Studies with Metabolites, Submitted under MRID Nos. 446327-03-04-05-06; and 446395-01

ID No. 066478-00002

P.C. Code: 121601 (003B)
Submission: S548078
D .P Barcode: D249059

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch 2
Health Effects Division (7509C)

10-28-98

THRU: Stephen C. Dapson, Ph.D., Senior Branch Scientist
Toxicology Branch 2
Health Effects Division (7509C)

Stephen C. Dapson 11/13/98

TO: Jim Thompkins/Phil Errico, PM E5
Registration Division (7505C)

REGISTRANT: Acetochlor Registration Partnership, c/o Zeneca Ag Products, Wilmington DE

REQUEST: Review and evaluate the following studies, submitted in response to the Agency's letter of July 10, 1997 stating that the ESA (sulphonic acid) and oxanilic acid degradation products may constitute residues of toxicological concern:

Study 1: Oxanilic Acid (R290130): Acute Oral Toxicity to the Rat, performed at (Zeneca's) Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire (UK), Study No. AR6414/Report No. CTL/P/5644, dated 25 September 1997. MRID 44632703. Unpublished.

Study 2: Sulphonic Acid (R290131): Acute Oral Toxicity to the Rat, performed at (Zeneca's) Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire (UK), Study No. AR 6415/Report No. CLT/P/5648, dated 17 November 1997. MRID 44632704. Unpublished.

Study 3: Oxanilic Acid (R290130): An Evaluation of Mutagenic Potential Using *S. typhimurium* and *E. coli*, performed at (Zeneca's) Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire (UK), Study No. YV 3984/Report No. CTL/P/5542, dated 4 August 1997. MRID 44632705. Unpublished.

Study 4: Sulphonic Acid (R290131): An Evaluation of Mutagency Potential Using *S. typhimuriumand E. coli*, performed at (Zeneca's) Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire (UK), Study No. YV 3985/Report No. CTL/P/5568, dated 4 August 1997. MRID 44632706. Unpublished.

Study 5: Acetochlor Soil Metabolites: Summary of Metabolism and Toxicology Studies, (Zeneca's) Laboratory Project ID BJK20, dated 14 August 1998. MRID 44639501. Unpublished.

TB CONCLUSIONS: These studies have been evaluated as follows (detailed reviews are attached).

	Study/Type/Chemical (MRID)	Reported Results	Evaluation
1.	Acute oral/oxanilic acid (44632703)	LD50 (males/females) greater than 2000 mg/kg (the limit dose). Tox. Cat. III	Acceptable
2.	Acute oral/sulphonic acid (44632704)	LD50 (males/females) greater than 2000 mg/kg (the limit dose) Tox. Cat. III	Acceptable
3.	Bacterial reverse gene mutation/oxanilic acid (44632705)	Negative for induced gene mutation at doses up to the limit, 5000 *u*g/plate, with/without activation.	Acceptable
4.	Bacterial reverse gene mutation/sulphonic acid (44632706)	Negative for induced gene mutation at dosses up to the limit, 5000 *u*g/plate, with/without activation.	Acceptable
5.	Summary of Studies (44639501)	(Several metabolism and acute toxicology studies conducted with the oxanilic and sulfonic acid metabolites are summarized)	[Not assessed; summary only]

EPA Reviewer: Irving Mauer, Ph.D.
Toxicology Branch 2, Health Effects Division (7509C)
EPA Secondary Reviewer: Stephen C. Dapson, Ph.D.
Toxicology Branch 2, Health effects Division (7509C)

Date: 10-28-98

Date: 11/13/98

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DATA EVALUATION RECORD

STUDY TYPE: Acute oral toxicity in the rat; OPPTS 870.1100 (81-1)

DP BARCODE: D249059

SUBMISSION CODE: S 548078

P.C. CODE: 121601

TOX. CHEM. NO.: 003B

TEST MATERIAL (PURITY): Oxanilic acid (metabolite of acetochlor, 97%)

SYNONYMS: R290130

CITATION: Lees, D. (1997). Oxanilic Acid (R290130): Acute Oral Toxicity to the Rat, performed at (Zeneca's) Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire (UK), Study No. AR6414/Report No. CTL/P/5644, dated 25 September 1997. MRID 44632703. Unpublished.

SPONSOR: Acetochlor Registration Partnership, c/o Zeneca Ag Products, Wilmington (DE)

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44632703), a single group of five male and five female Alpk:AP$_f$SD (Wistar-derived) rats received a single oral dose of 2000 mg/kg test article. Animals were observed daily for 14 days for signs of clinical toxicity and body weight was recorded periodically throughout the study. At the end of the observation period, all animals were killed and examined macroscopically.

There were no deaths and none of the animals manifested or clinical macroscopically adverse findings; all showed overall weight gain during the study. Therefore, the acute median lethal dose (LD50) of oxanilic acid is estimated to be greater than 2000 mg/kg, the limit dose for this type of study. Tox. Cat. assigned is III.

This acute oral study is classified Acceptable-Guideline and satisfies the requirement for an acute oral study (81-1) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data confidentiality, and flagging statements were provided.

I. MATERIALS AND METHODS

A. Materials:

1. Test Material: Oxanilic acid (R290130)
   Description: White solid
   Lot/Batch No.: OSW 01738-OIR
   Purity: 97% a.i.
   CAS No.: [Not provided]
   Verification of concentration/homogeneity: Not provided

2. Vehicle and/or positive control:
   Corn oil
   Positive control: None.

3. Test animals: Species: Rat
   Strain: Alpk:AP$_f$SD
   Age and/or weight at dosing: 8-12 weeks/males: 288-329 g; females: 217-244g.
   Source: Rodent Breeding Unit, Alderley Park, Macclesfield, Cheshire (UK)
   Acclimation period: Five days
   Diet: R&M No.1 ad libitum
   Water: Mains ad libitum
   Environmental conditions:
   Temperature, 22±30 degrees C
   Humidity, 30-70%
   Air changes, 15+ per hour
   Photoperiod: 12 hours light/12 hours dark

B. STUDY DESIGN AND METHODS:

1. In life dates: start: 9 June/end: 24 June 1997

2. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 1. Following an overnight fast, rats were given a single dose of 2000 mg/kg by gavage, then observed daily for 14 days, and weighed prior to fasting (day -1), immediately before dosing (day 1) and on days 8 and 15. Survivors were sacrificed and a necropsy was performed.

3. Statistics: The oral LD50 was not calculated since no deaths occurred at the limit dose, 2000 mg/kg.

Table 1. Doses, Mortality/Animals Treated			
Dose (mg/kg)	Males	Females	Combined
2000	0/5	0/5	0/10

II. RESULTS AND DISCUSSION

A. Mortality is given in Table 1, above (derived from MRID 44632703 Report, page 18, attached). The oral LD50 for males and females is greater than 2000 mg/kg (the limit dose for this type of study).

B. Clinical observations: No adverse clinical abnormalities observed (MRID 44632703 Report Table 2, pages 19-23).

C. Body weight: Overall body weight gain throughout study (MRID 44632703 report Table 3, page 24).

D. Necropsy: No treatment-related macroscopic findings (MRID 44632703 Table 4, pages 25-34).

E. Deficiencies: [None]

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY -- SEE THE FILE COPY

ATTACHMENT

TABLE 1 - CUMULATIVE MORTALITY DATA

Dose level (mg/kg)	Day Number	Number of Deaths	
		Male	Female
2000	-	0	0
	15 (total)	0/5	0/5

EPA Reviewer: Irving Mauer, Ph.D.
Toxicology Branch 2, Health Effects Division (7509C)
Secondary EPA Reviewer: Stephen C. Dapson, Ph.D.
Toxicology Branch 2, Health Effects Division (7509C)

Date: 10-28-98

Date: 11/13/98

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DATA EVALUATION RECORD

STUDY TYPE: Acute oral toxicity in the rat, OPPTS 870.1100 (81-1)

DP BARCODE: D249059 SUBMISSION CODE: S 548078

P.C. CODE: 121601 TOX. CHEM. NO.: 003B

TEST MATERIAL (PURITY): Sulphonic acid (ESA metabolite of acetochlor, 97%)

SYNONYMS: R290131

CITATION: Lees D. (1997) Sulphonic Acid (R290131): Acute Oral Toxicity to the Rat, performed at (Zeneca's) Central Toxicology Lab, Alderley Park, Macclesfield, Cheshire (UK), Study No. AR 6415/Report No. CTL/P/5648. MRID 44632704. Unpublished.

SPONSOR: Acetochlor Registration Partnership, c/o Zeneca Ag Products, Wilmington (DE)

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44632704), a single group of five male and five female Wistar-derived rats received a single oral dose of 2000 mg/kg test article, and were observed daily for 14 days, with periodic body weight measurement throughout the study. At termination, all animals were necropsied and subjected to a macroscopic examination post-mortem.

No animals died, and there were no treatment-related clinical signs, necropsy findings or significant changes in body weight. Tox. Cat. assigned is III.

This acute oral study is classified Acceptable-Guideline and satisfies the Guideline requirement for an acute oral study (81-1) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Sulphonic acid (R290131)
   Description: White solid
   Lot/Batch No.: ASW 01741-01R, TSC 0452/03623
   Purity: 97% a.i.
   CAS No.: [Not provided]
   Verification of concentration/homogeneity: Not provided.

2. Vehicle and/or positive control:
   Deionized Water (DW) [No positive control.]

3. Test animals: Species: rat
   Strain: Alpk:AP$_f$SD
   Age and/or weight at dosing: 8-12 weeks; males 292-346g /females 202-236 g.
   Source: Rodent Breeding Unit, Alderley Park, Mecclesfield, Cheshire (UK)
   Diet: R&M No. 1 ad libitum
   Water: ad libitum
   Environmental Conditions:
   Temperature: 22±3 degrees C
   Humidity: 30-70%
   Air changes: 1.5+ per hour
   Photoperiod: 12 hours light/12 hours dark

B. STUDY DESIGN and METHODS:

1. In life dates - start: 16 June/end: 10 July 1997

2. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 1. Following an overnight fast, rats were given a single dose of 2000 mg/kg by gavage, then observed and weighed (periodically) for 14 days. Survivors were sacrificed and a necropsy was performed.

Dose (Mg/kg)	Males	Females	Combined
2000	0/5	0/5	0/10

3. Statistics: The oral LD50 was not calculated since no deaths occurred at 2000 mg/kg, the limit dose (see below).

II. RESULTS AND DISCUSSION:

A. Mortality is given in Table 1 (derived from MRID 44632704 Table 1, Report page 18, attached). The oral LD50 for males and females is greater than 2000 mg/kg.

B. Clinical observations - There were no signs of clinical toxicity (MRID 46632704 Table 2, Report pages 19-23).

C. Body weight - All animals gained body weight during the study. (MRID 44632704 Table 3, Report page 24).

D. Necropsy - There were no compound-related findings at the examination post-mortem. (MRID 44632704, Table 4, Report pages 25-34).

E. Deficiencies - There were no deficiencies.

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ATTACHMENT

TABLE 1 - CUMULATIVE MORTALITY DATA

Dose level (mg/kg)	Day Number	Number of Deaths	
		Male	Female
2000	-	0	0
	15 (total)	0/5	0/5

EPA Reviewer: Irving Mauer, Ph.D. Date: 10-28-98
Toxicology Branch 2, Health Effects Division (7509C)
EPA Secondary Reviewer: Date: 11/13/98
Toxicology Branch 2, Health Effects Division (7509C)

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DATA EVALUATION RECORD

STUDY TYPE: Bacterial systems (Salmonella, E. coli)/mammalian activation gene mutation assay; OPPTS 870.5100 (84-2)

DP BARCODE: D 249059 SUBMISSION CODE: S 548078

P.C. CODE: 121601 TOX. CHEM. NO.: 003B

TEST MATERIAL (PURITY): Oxanilic acid (R290130, acetochlor metabolite, 97%)

SYNONYMS: [None]

SPONSOR: Acetochlor Registration Partnership, c/o Zeneca Ag Products, Wilmington (DE)

CITATION: Callander, R.D. (1997) Oxanilic Acid (R290130): An Evaluation of Mutagenic Potential Using S. typhimurium and E. coli, performed at (Zeneca's) Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire (UK), Study No. YV 3984/Report No. CTL/P/5542, dated 4 August 1997. MRID 44632705. Unpublished.

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 44632705), four mutant (*his*$^-$) strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and two mutant (try$^-$) strains of *Escherichia coli* (WP2P and WP2P *uvr*A) were exposed in two separate trials to the oxanilic acid metabolite of acetochlor (97% a.i.) in the presence and absence of a rat liver-derived metabolic activation system (S9-Mix). Solvent controls and strain-specific mutagens were included in each trial.

The test material was tested up to 5000 *u*g /plate (the limit concentration) without the induction of any significant reproducible increases in the frequency of revertant colonies (*his*+,*try*+) under either condition of activation (± S9)in either trial. Positive controls gave the expected mutagenic responses. Hence, oxanilic acid is non-mutagenic in this bacterial assay.

This study is classified as Acceptable-Guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

1. Test Material: Oxanilic acid (R290130)
Description: White solid.
Lot/Batch No. ASW01738-01R
Purity: 97% a.i.
Stability of compound: Not performed, but Certificate of Analysis (dated March 7, 1997) provided.
CAS No.: [Not provided]
Solvent used: Sterile deionized water

2. Control Materials:

Negative: [Not included]
Solvent/final concentration: 100 *u*L/plate
Positive: Non-activation:
Sodium azide: 0.5, 1.0, 2.0 *u*g/plate for TA100, TA1535
Daunomycin HCl: 0.2, 0.5, 1.0 *u*g/plate for TA98
ICR-191 (acridine mutagen): 0.5, 1.0, 2.0 *u*g/plate for TA1537

Other (list):
Mitomycin-C, 0.2, 0.5, 1.0 *u*g/plate for WP2P
EENG: 0.2, 0.5, 1.0: 0.2, 0.5, 1.0 *u*g/plate for WP2P *u*vrA

Activation:

2-Aminoanthracene (2-anthramine)
0.2, 0.5, 1.0 *u*g/plate for TA98, TA100,
0.5, 1.0, 2.0 *u*g/plate for TA1535, TA1537
5, 10, 20 *u*g/plate for WP2P
1, 2, 5 *u*g/plate for WP2P *u*vrA

3. Activation: S9 derived from male Sprague-Dawley rats.

	Aroclor 1254	x	induced	x	rat		liver
x	phenobarbital/ naphthaflavone		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition:
S9 fraction: 3 mL
Sucrose - Tris-EDTA buffer: 7 mL
Co-factor solution (Na_2HPO_4, KCl,G-6-P, NADP, $MgCl_2$): 20 mL

4. Test organisms: *S. typhimurium* strains:

	TA97	x	TA98	x	TA100		TA102		TA104
x	TA1535	x	TA1537		TA1538				

List any others: *E. coli* strains: WP2P and WP2P *uvr*A.

Properly maintained: Yes.

Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test compound concentrations used

Non-activated conditions: 100, 200, 500, 1000, 2500, 5000 *u*g/plate.
Activated conditions: 100, 200, 500, 1000, 2500, 5000 *u*g/plate.

B. TEST PERFORMANCE:

1. Type of Salmonella assay:

X___ standard plate test (initial trial)
X___ pre-incubation (60 minutes in second trial)
_____ "Prival" modification
_____ Spot test
_____ other (describe)

2. Protocol: Cultures of each strain were exposed to the above range of concentrations of test article, (three plates per concentration), or to 100 *u*l/plate solvent (5 plates), or in duplicate to above concentrations of reference mutagens, and incubated at 37 degrees C for three days. Revertant colonies were counted (by Automatic Colony Counter) and compared to solvent control values.

II. REPORTED RESULTS

Mutagenicity assay: (only) None of the cultures in either assay showed significant, reproducible (greater than twofold) increases over solvent controls in number of revertant colonies (Report Tables 1-4, pp. 21-24, attached). Therefore the investigator concluded that oxanilic acid was negative for induced mutagenicity in these bacterial strains under the conditions of this assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. We agree with the author that under the conditions of these assays, oxanilic acid is non-mutagenic in the strains of *Salmonella* and *E. coli* tested.

B. STUDY DEFICIENCIES: There are no deficiencies that would compromise the conclusions or assessment of this study.

Acute tox Review MRID's 44632703 thru 06

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____ Identity of product impurities.

____ Description of the product manufacturing process.

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EPA Reviewer: Irving Mauer, Ph.D
Toxicology Branch 2, Health Effects Division (7509C)
EPA Secondary Reviewer:
Toxicology Branch 2, Health Effects Division (7509C)

Date: 10-28-98

Date: 11/13/98

013221

DATA EVALUATION RECORD

STUDY TYPE: Bacterial systems, (*Salmonella/E. coli*)/mammalian activation gene mutation assay; OPPTS 870.5100 (84-2)

DP BARCODE: D249059 SUBMISSION CODE: S548078

P. C. CODE: 121601 TOX. CHEM. NO.: 003B

TEST MATERIAL (PURITY): Sulphonic acid (R290131, acetochlor metabolite, 97%)

SYNONYMS: [None]

CITATION: Callander, R.D. (1997) Sulphonic Acid (R290131): An Evaluation of Mutagenic Potential Using *S. typhimurium* and *E. coli* , performed at (Zeneca's) Genetic Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire (U.K.), Study No. YV3985/Report No. CTL/P/5568, dated 4 August 1997. MRID 44632706. Unpublished.

SPONSOR: Acetochlor Registration Partnership, c/o Zeneca Ag Products, Wilmington (DE)

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 4432706), four mutant (*his*$^-$) strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and two mutant (*try*$^-$) strains of *Escherichia coli* (WP2P and WP2P *uvr*A) were exposed in two separate trials to the sulphonic acid metbolite of acetochlor (97% a.i.), in the presence and absence of a rat liver-derived metabolic system (S9-Mix). Solvent controls and strain-specific mutagens were included in each trial.

The test material was tested up to 5000 *u*g/plate (the limit concentration), without the induction of any significant, reproducible increase in the frequency of revertant colonies (*his*$^+$, *try*$^+$) under either condition of activation (±S9) in either trial. Positive controls responded with the expected increase in revertants. Hence sulphonic acid is considered non-mutagenic in this bacterial assay.

This study is classified as Acceptable-Guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Sulphonic acid (R290131)
Description: White solid
Lot/Batch No. ASW 01741-01R
Purity: 97% a.i.
Stability of compound: Not performed, but Certificate of Analysis (dated March 3, 1977) provided.
CAS. No.: [Not provided]
Solvent used: sterile deionized water

2. Control Materials:
Negative: [Not included]
Solvent/final concentration: 100 *u*L/plate
Positive: Non-activation:
Sodium azide: 0.5, 1.0, 2.0 *u*g/plate for TA100, TA1535
Daunomycin HCl: 0.2, 0.5, 1.0 *u*g/plate for TA98
1CR191 (acridine mutagen): 0.5, 1.0, 2.0 *u*g/plate for TA1537
Other (list):
Mitomycin C: 0.2, 0.5, 1.0 *u*g/plate for WP2P
EENG: 0.2, 0.5, 1.0 *u*g/plate for WP2P *uvrA*

Activation:
2-Aminoanthracene (2-anthramine):
0.2, 0.5, 1.0 *u*g/plate for TA98, TA100
0.5, 1.0, 2.4 *u*g/plate for Ta 1535, TA1537
5, 10, 20 *u*g/plate for WP2P
1, 2, 5 *u*g/plate for WP2P *uvrA*

3. Activation: S9 derived from male Sprague-Dawley rats.

	Aroclor 1254	x	induced	x	rat	x	liver
x	phenobarbital/ naphthalflavone		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition:
S9 fraction: 3mL
Sucrose-Tris-EDTA buffer: 7 mL
Cofactor solution: (Na_2HPO_4, KCl, G-6-P, NADP, $MgCl_2$): 20 mL

4. Test compound concentrations used:

Non-activated conditions: 100, 200, 500, 1000, 2500, 5000 *u*g/plate
Activated conditions: 100, 200, 500, 1000, 2500, 5000 *u*g/plate

B. TEST PERFORMANCE

1. Type of Salmonella assay:

X___ standard plate test (initial trial)
X___ pre-incubation (60 minutes, i.e., second trial)
_____ "Prival" modification
_____ spot test
_____ other (describe)

2. Protocol: Cultures of each strain were exposed to the above range of test article concentrations (three plates per concentration), or to 100 *u*l/plate solvent (5 plates), or in duplicate to the above concentrations of reference mutagens, and incubated at 37 degrees C for three days. Revertant colonies were counted (by Automatic Colony Counter) and compared to solvent control values.

II. REPORTED RESULTS

Mutagenicity assay (only): Except for slight but significant increases in TA1537 test cultures in the first plate incorporation assay under S9 activation, none of the other test cultures in either assay showed significant reproducible increases (greater than two-fold) over solvent controls in the number of revertants (Report Tables 1-4, pp. 21-24). The increases noted in TA1537 were not reproducible in the pre-incubation trial (Table 2) nor in the second plate incorporation experiment (Table 4), hence were not considered biologically relevant. The investigator concluded that sulphonic acid was negative for induced mutagenicity in these bacterial strains under the conditions of this assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. We agree with the investigator that sulphonic acid was not mutagenic in the strains of *Salmonella* and *E. coli* tested under the conditions of this assay.

B. STUDY DEFICIENCIES:

There were no deficiencies that would compromise the conclusions or assessment of this study.

Acute tox Reviews MRID's # 44632703 thru 06

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