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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

Subject: EPA ID# 113201: Vinclozolin, DER for the Second Dermal Developmental Toxicity Study in Rats.

Barcode: D217291. PC No.: 113201.
Submission No.: S490294. Rereg. Case No.: 2740.
MRID No.: 43703301. Case No.: 011409.
ToxChem No.: 323C. CAS No.: 50471-44-8.

From: David G Anderson, PhD 12/19/95
Toxicologist,
Section 3, Toxicology Branch-1
HED (7509C)

To: Bruce Sidwell/Mark Wilhite, PM 53
Accelerated Reregistration Branch
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Thru: Karen Hamernik, PhD 12/20/95 12/20/95
Section 3 Head, Toxicology Branch-1
HED (7509C)

BASF submitted a Second dermal developmental toxicity study with vinclozolin to establish a NOEL in a study extending the dose period closer to parturition.

Hellwig, J (April 27, 1995) Study of the Prenatal Toxicity of Reg No. 83 258 in Wistar Rats After Dermal Application, Study report# 34R0375/88124 and BASF# 95/10450. Study conducted at BASF Crpo Protection, Product Safety, Dept. Toxicology, BASF, Germany for BASF AG Corp., RTP, NC. MRID# 43703301.

EXECUTIVE SUMMARY: In a dermal developmental toxicity study (MRID# 43703301), Vinclozolin, 99.3% a.i. was administered to 24-25 pregnant female Wistar rats, Chbb:Thom (SPF) per group via the skin in 0.5% carboxymethylcellulose in water at dose levels of 0, 10, 20, 30 or 200 mg/kg/day from gestational day 6 through 20.

No dose related maternal toxicity in organ weights or body weights were reported. No mortality or dose related clinical signs, body weight gain decrement were reported in dams. Only food consumption was statistically significantly reduced on gestational day (gd) 13-15 at the 2 top dose levels and 15-17 at 200 mg/kg/day. The reduction in food consumption may have been incidental since body weight gain was not affected.

The only possible effects reported was a nominal decrease in

the ano-genital-distance (AGD) (6%) and ano-genital-index (AGI) (5%) at nominal dose of 200 mg/kg/day. Since the analytical dose level was 144-169 mg/kg/day at the 200 mg/kg/day nominal dose level, the dose level may have been insufficient for the AGD and AGI to attain the statistical significance that was seen in first dermal developmental toxicity study at 180 and 360 mg/kg/day. In addition, in this study AGD was determined on unfixed fetuses, while AGD was determined on fixed fetuses in the first dermal developmental study that showed a smaller standard deviation in the AGD and the AGI measurements. The standard deviations in males in controls were about 2-3 times the values in control males in the first dermal developmental toxicity study (MRID# 41413001).

The serum levels of vinclozolin and the metabolites/ degradation products M1 and M2 were determined in dams and fetuses at termination. Vinclozolin and M2 were not detected, but M1 was 4.32 nmoles/g-serum in dams and 7.00 nmoles/g-serum in fetuses at the HDT, only.

The maternal LEL > 144-169 mg/kg/day (no NOEL) was not determined based on no dose related effects at the analytical HDT, nominal 200 mg/kg/day. The developmental LEL/NOEL is a threshold based on nominally ($p \geq 0.05$) decreased AGD or AGI at the analytical HDT of 144-169 mg/kg/day. The developmental NOEL in this study can not be accurately assessed based on these effects

REGULATORY COMPLIANCE: The developmental toxicity study in the rat is classified acceptable and satisfies the guideline requirement for a dermal developmental toxicity study (OPPTS 870.3700; §83-3b) in the rat. However, the 144-169 mg/kg/day NOEL is considered a threshold because of the increased variability of AGD and AGI and analytical dose levels at the HDT, therefore, the NOEL of 60 mg/kg/day from the first dermal developmental toxicity study (MRID# 41413001, HED Doc.# 007870 and 008556) should be used for appropriate dermal risk assessment.

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[Vinclozolin] Developmental Study (83-3b)

EPA Reviewer: David G Anderson, PhD. David G Anderson 12/19/95
Review Section 3, Toxicology Branch-1 (7509C)
EPA Section Head: Roger L Gardner, PhD. R.L. 12/20/95
Review Section 1, Toxicology Branch-1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Second Dermal Developmental Study - Rats (83-3)

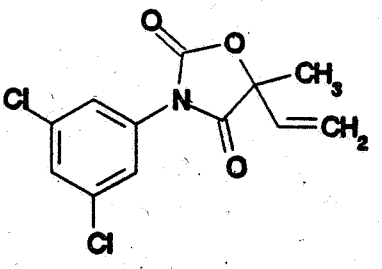
DP Barcode No.: D217291.
Submission No.: S490294.
ToxChem. No.: 323C.
PC No.: 113201.

Cas No.: 50471-44-8.
Action Code: 627.
Rereg. Case No.: 2740.
Case: 816411.
MRID No.: 43703301.

TEST MATERIAL: Reg No. 83 258, [Vinclozolin], purity 99.3%.

SYNONYMS: [[3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedi-2,3-one], Ronilan™.

STRUCTURE:



STUDY REPORT NUMBER(S): Lab. Proj.# 34R0375/88124. BASF# 95/10450.

SPONSOR: BASF AG Corp., Res. Triangle Park, NC.

TESTING FACILITY: BASF Aktiengesellenschaft Crop Protection, Product Safety, Dept. Toxicology, D-67056 Ludwigshafen/Rhein, Germany.

TITLE OF REPORT: Study of Prenatal Toxicology of Reg. No. 83 258 in Wistar Rats After Dermal Application.

AUTHOR(S): Hellwig, J.

REPORT ISSUED: April 27, 1995.

EXECUTIVE SUMMARY: In a dermal developmental toxicity study (MRID# 43703301), Vinclozolin, 99.3% a.i. was administered to 24-25 pregnant female Wistar rats, Chbb:Thom (SPF) per group via the skin in 0.5% carboxymethylcellulose in water at dose levels of 0, 10, 20, 30 or 200 mg/kg/day from gestational day 6 through 20.

No dose related maternal toxicity in organ weights or body weights were reported. No mortality or dose related clinical signs, body weight gain decrement were reported in dams. Only food consumption was statistically significantly reduced on gestational day (gd) 13-15 at the 2 top dose levels and 15-17 at

200 mg/kg/day. The reduction in food consumption may have been incidental since body weight gain was not affected.

The only possible effects reported was a nominal decrease in the ano-genital-distance (AGD) (6%) and ano-genital-index (AGI) (5%) at nominal dose of 200 mg/kg/day. Since the analytical dose level was 144-169 mg/kg/day at the 200 mg/kg/day nominal dose level, the dose level may have been insufficient for the AGD and AGI to attain the statistical significance that was seen in first dermal developmental toxicity study at 180 and 360 mg/kg/day. In addition, in this study AGD was determined on unfixed fetuses, while AGD was determined on fixed fetuses in the first dermal developmental study that showed a smaller standard deviation in the AGD and the AGI measurements. The standard deviations in males in controls were about 2-3 times the values in control males in the first dermal developmental toxicity study (MRID# 41413001).

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Signed and dated GLP inspections 8/1/1994 and 1/17/1995 and Quality Assurance statements, Data Confidentiality, and Flagging Statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: [Reg. No. 83 258, technical, vinclozolin]
   Description (a technical grade white solid):
   Lot/Batch #: N 183
   Purity: 99.3% a.i.
   Stability of compound: Stable for the dosing period.

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2. Vehicle: 0.5% carboxymethylcellulose in water, (Tylose™ CB 30,000, purified).

3. Test animals: Species: Rat
Strain: Wistar Chbb:THOM (SPF)
Age and weight at study initiation: Age is 76 to 90 days; the mean body weight of groups were 238 g at gestational day (gd) 0.
Source: Thomae GmbH, Biberauch an der Riss, FRG.
Housing: DK III stainless steel wire mesh cages, 800 cm^2.
Diet - Animals were given Kliba 343 feed for the rat/mouse/hamster and water *ad libitum*.
Environmental conditions: Temperature: 20-24°C.
Humidity: 30-70%.
Air changes: Not presented.
Photoperiod: 12 hr light: 12 hr dark.
Acclimation period: 5 days.

B. PROCEDURES AND STUDY DESIGN:

This study was designed to assess the developmental toxicity potential of vinclozolin when administered dermally to rats on gestation days 6 through 20, inclusive. The animals were clipped prior to application, covered with gauze and wrapped with rubberized material. The test material was applied daily and removed daily after 6 hours and washed. Blood was drawn from 5 dams and their fetuses per group on gestational day (gd) 21 to determined levels of vinclozolin, M1 and M2. Previous studies had established not dose related skeletal of visceral anomalies below 360 mg/kg/day (MRID# 41413001, HED Doc.# 007576 and 008556), therefore, only external examination was conducted and visceral examination was studied for sex determination. However, malformations and anomalies noted were recorded.

1. Mating: Mating (1:1) was natural.

2. Animal Assignment and dose selection is presented in (Table 1). Assignment was random.

Table 1: Animal assignment.

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	25
Low (LDT)	10	25
Mid1 (MDT1)	20	25
Mid2 (MDT2)	30	25
High (HDT)	200	25

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3. Dose selection rationale:
Dosing was stopped in the first dermal developmental toxicity study on gd 19. Dosing in this second dermal developmental toxicity study extended as long as possible to gd 20 because an oral perinatal developmental toxicity study indicated that the oral NOEL had been reduced from 15 mg/kg/day to 3 mg/kg/day by extending dosing to post-natal day 3. The NOEL/LEL were 60/180 mg/kg/day in the first dermal study (MRID# 41413001, HED Doc.# 007576 and 008556). Lower doses were included to determine a possible lower NOEL analogous to the lower oral NOEL.

4. Dosage preparation and analysis: The dosing solutions were analyzed for concentration and homogeneity twice during the study. The test material has been shown to be stable and homogenous under dosing conditions in the first dermal developmental toxicity study (MRID# 41413001). The homogeneity was satisfactory and concentration (85% to 105%) was satisfactory at the 3 lower dose levels. In addition, the percentage of nominal for deep frozen samples (-20°C) were a satisfactory 93% to 102% at 50 mg/kg/day, 92% to 103% at 100 mg/kg/day, 100% to 104% at 300 mg/kg/day and 100% to 104% at 200 mg/kg/day. For these samples the same concentration was used in the dosing suspensions for the 200 mg/kg/day dose level.

However, at the HDT, the 2 retained samples from the 200 mg/kg/day suspension were analyzed and the concentration found to be 71.8% and 84.5% of nominal. Other samples presented on page 0229 of study number 95/10450, the submitted report, were not identified with respect to their relationship with the dosing suspensions (i.e., whether they simulations of the dosing suspensions or samples of the dosing suspensions). These latter samples yielded even lower analytical concentrations of 46.4% and 67.5% of nominal for the 200 mg/kg/day dose level sample. These latter results would yield 92.8 and 135.6 mg/kg/day for the 200 mg/kg/day dose level sample. The analytical dose levels for the retained samples for the nominal dose level of 200 mg/kg/day were 144 and 169 mg/kg/day. These latter values were considered relevant to the dosing suspensions used in the study by the registrant.

Results - Homogeneity Analysis: 85% to 105% of nominal. Stability Analysis: 90.9% to 104.3% for 3 hours. Concentration Analysis: 95.3% to 104.8% of nominal for the 10 and 20 mg/kg/day samples and 108.3% to 108.9% for the 30 mg/kg/day samples and 71.8% to 84.5% for the 200 mg/kg/day samples.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable for the 10, 20 and 30 mg/kg/day dose levels. However, the 200

mg/kg/day dose levels was considered to be 144 to 169 mg/kg/day and may have been as low as 92.8 or 135.6 mg/kg/day.

5. Dosage adminstration: All doses were administered for 6 hours daily to the skin in a volume of 5 ml/kg of body weight/day, based on the most recent body weight.

C. OBSERVATIONS:

1. Maternal Observations and Evaluations - The animals were checked for mortality or clinical signs daily. Body weight and food consumption data were recorded on gd 0, 1, 3, 6, 8, 10, 13, 15, 17, 20 and 21. Dams were sacrificed on day 21 of gestation. Examinations at sacrifice consisted of: post mortem (gross pathological examination), body weight, uterine weight, implantation sites by the Salewski method (early and late), corpora lutea, viable fetuses and dead fetuses.

2. Fetal Evaluations - The fetuses were examined in the following manner: number viable fetuses, fetal weight, external examination, sex determination by visceral examination and ano-genital distance (AGD) and index, conducted in a blind fashion.

D. DATA ANALYSES: The following statistical analysis methods were employed: Dunnett-test - for food consumption, body weight and gain, fetal weight, corpora lutea, implantations, pre and post -implantations, live dead fetuses, and ano-genital-distance and index. Fisher Exact test - for number pregnant, mortality and number of fetuses with findings. Wilcoxon test - proportion of fetuses with findings per litter.

1. Indexes: The only defined index in the report was the ano-genital-index (AGI) = (AGD/fetal-weight) X 100. The ano-genital-distance (AGD) = the distance in mm from the anus to the genital tubercle.

2. Historical control data: Histroical control data were provided to allow comparison with concurrent controls.

II RESULTS

A. MATERNAL TOXICITY

1. Mortality - None

2. Clinical Observations - No dose related observations were seen.

3. Body Weight - Body weight data are summarized in Table 2 and as follows: No dose related decrements were reported for maternal body weights or gains.

Table 2: Maternal body weights and body weight gains (g)[a]

INTERVAL	DOSE in mg/kg/day (# of DAMS)				
	0 (25)	10 (25)	20 (24)	30 (25)	200 (25)
GD 0	239.7	234.4	240.8	239.1	237.8
GD 6	263.1	260.9	264.9	264.5	262.8
GD 8	264.6	262.1	265.8	266.3	265.8
GD 21	375.0	369.0	375.3	378.7	377.2
Carcass weight	286.0	284.2	289.0	291.2	289.5
PRETREATMENT: Days 0-6	23.4	26.5	24.1	25.4	25.0
TREATMENT: Days 6-8	1.5	1.2	0.9	1.8	3.0
Days 8-20	99.8	95.9	97.8	101.8	103.4
POSTTREATMENT Days 20-21	12.1	12.2	12.6	12.4	11.0

[a] = Data extracted from study report# BASF 95/10450, Table# 003, 004, 005 & 006, page 0059, 0060, 0061 & 0062. *, ** = Statistically significant at p≤ 0.05 and p≤ 0.01, respectively.

4. Food Consumption - Food consumption data are summarized as follows: Food consumption was statistical significantly increased 6.3% only gd 13-15 at 30 and 200 mg/kg/day and 6.7% on gd 15-17 at 200 mg/kg/day (Study report# 95/10450, Table 001, page 0057). Since no corresponding decreased occurred in body weight gain, the effect on food consumption may have been incidental.

5. Gross Pathology - Gross pathology data are summarized as follows: No dose related gross pathology was noted. Organ weight were not determined.

6. Cesarean section Data - No dose related malformations were reported (Table 3). Although full visceral and skeletal determination were not conducted nor necessary in the current study, they were conducted in the first dermal developmental toxicity study (MRID# 41413001).

Table 3: Cesarean Section Observations[a]

Dosages (mg/kg/day)	0 (cont.)	10 (LDT)	20 (MDT1)	30 (MDT2)	200 (HDT)
# Animals Assigned	25	25	25	25	25
# Animals Mated/pregnant	25	25	24	25	25
Pregnancy Rate (%)	100	100	96	100	100
Maternal Wastage # Died # Died/Pregnant # Non Pregnant #Aborted # Premature Delivery	0 0 0 0 0 0	0 0 0 0 0 0	1 0 0 0 0 0	0 0 0 0 0 0	0 0 0 0 0 0
Corpora Lutea/Dam	15.3	15.0	15.0	15.6	14.5
Implantations/Dam	13.6	13.2	14.0	13.8	13.2
Total live fetuses	315	303	296	310	311
Live Fetuses/Dam	12.6	12.1	12.3	12.4	12.4
Early	0.8(5.3)	1.0(7.0)	1.2(8.5)	1.0(6.7)	0.6(3.7)
Late	0.2(1.4)	0.1(0.6)	0.5(4.0)	0.4(2.3)	0.2(1.8)
Resorptions/Dam	1.0(6.7)	1.1(7.7)	1.7(12.4)	1.4(9.0)	0.8(5.5)
Total Dead Fetuses	0	0	0	0	0
Dead Fetuses/Dam	0	0	0	0	0
Mean Fetal Weight (g) Males Females	5.2 5.4 5.1	5.2 5.3 5.1	5.2 5.4 5.1	5.3 5.4 5.2	5.3 5.4 5.1
Preimplantation Loss (%)	11.0	11.8	6.4	12.4	10.1
Postimplantation Loss (%)	6.7	7.7	12.4	9.0	5.5
Sex Ratio (% Male)	48.3	53.1	50.7	42.6	55.0

[a] = Data extracted from study report# BASF 95/10450, Table 009, 010, 011 & 013, page 0065, 0066, 0067 & 0069). *, ** = Statistically significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

B. DEVELOPMENTAL TOXICITY:

1. External Examination - Nominally reduced ano-genital-distance (AGD) and ano-genital-index AGI (Table 4) at the

HDT. AGD was reduced 6% at the HDT and AGI was reduced 5% from control values at the HDT in males. Males were identified by visceral examination. Neither the AGD or the AGI were statistically significantly different from control values. Females were not affected. No dose related anomalies were seen (Table 5) other than the random malformations seen at all dose levels.

Table 4: Ano-genital-distance (AGD) in mm[a].

Dose level (mg/kg/day)		0	10	20	30	200
Number of dams		25	25	24	25	25
All fetuses	Mean SD	2.4 0.32	2.4 0.34	2.4 0.21	2.2 0.28	2.4 0.29
Male fetuses	Mean SD	3.3 0.30	3.2 0.27	3.3 0.21	3.2 0.33	3.1 0.22
Females fetuses	Mean SD	1.5 0.13	1.4 0.11	1.5 0.12	1.5 0.15	1.5 0.10
Ano-genital-index (AGD/fetal weight)						
All fetuses	Mean SD	0.45 0.062	0.45 0.070	0.45 0.044	0.42 0.067	0.45 0.051
Males fetuses	Mean SD	0.61 0.066	0.60 0.062	0.61 0.053	0.60 0.072	0.58 0.051
Female fetuses	Mean SD	0.30 0.031	0.29 0.026	0.30 0.027	0.29 0.034	0.29 0.026

[a] = Data extracted from study# BASF 95/10450, Table 012 & 013, page 0068 & 0069.

Table 5: Findings on external examination[a].

Dose levels (mg/kg/day)	0	10	20	30	200
#Fetus(litters) examined	315(25)	303(25)	296(24)	310(25)	311(25)
#Fetuses(litters) affected					
Anasarca	0(0)[b]	2(2)	0(0)	0(0)	0(0)
Shortened upper jaw	1(1)	0(0)	0(0)	0(0)	0(0)
Cleft palate	0(0)	1(1)	0(0)	0(0)	0(0)
Shortened toes	0(0)	1(1)	0(0)	0(0)	0(0)
Filiform tail	0(0)	0(0)	0(0)	0(0)	1(1)

[a] = Data extracted from study report# BASF 95/10450, Tables 0015 & 0016, page 0071 & 0072.

[b] = fetal (litter) incidence

2. Visceral Examination - The visceral examination was primarily to establish the sex of the fetus. No dose related effects were seen.

3. Skeletal Examination - No skeletal examination was conducted.

4. Serum Levels of Vinclozolin, M1 and M2 in Dams and Fetuses - Serum levels of vinclozolin (Reg. No. 83 258), M1 and M2 were determined in 5 dams and 5 litters per groups at termination on gd 21 (See the Appendix for the stuctures, page 0030 of the submiited report 95/10450). Only M1 (the oxazolidine hydrolyses yielding a carboxylic acid metabolite/degradation product) was detected in dams and fetuses at the HDT. The values are 4.32 for dams and 7.0 nmoles/g-serum for fetuses (Table 6). The slightly higher value in fetuses than dams may be real and indicate why fetuses are affected with this low binding constant androgen receptor inhibitor.

Table 6: Serum levels of vinclozolin, M1 and M2 in dams and fetuses

Dose level (mg/kg/day	0	10	20	30	200
Levels of vinclozolin, M1 and M2 in dams (standard deviation).					
Vinclozolin	ND	ND	ND	ND	ND
M1 in nmoles/g-serum	ND	ND	ND	ND	4.32(1.09)
M1 in µg/g-serum	ND	ND	ND	ND	1.31
M2	ND	ND	ND	ND	ND
Levels of vinclozolin, M1 and M2 in fetuses (standard deviation).					
Vinclozolin	ND	ND	ND	ND	ND
M1 in nmoles/g-serum	ND	ND	ND	ND	7.00(1.94)
M1 in g/g-serum	ND	ND	ND	ND	2.13
M2	ND	ND	ND	ND	ND

[a] = Data was extracted form study report# BASF 95/10450, Figure 4.4.1.2., page 0049.
ND = Not detectable (limit of detection: vinclozolin-3 nmoles/g-serum; M1-2 nmoles/g-serum; M2-2 nmoles/g-serum). M1 is the hydrolysed product (oxazolidine ring is hydroylysed to the carboxylic acid sufficiently rapidly that vinclozolin is not detected). M1 inhibits androgen binding about an order of magnitude lower than M2. M2 is the enol metabolite that inhibits

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androgen binding within an order of magnitude lower than flutamide. 41756

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: Nominal effects on the AGD and AGI occurred at the HDT, was the analytical dose level of 144-169 mg/kg/day. However, the 60 mg/kg/day dose level from the first dermal developmental toxicity study should be considered the NOEL for appropriate risk assessment.

B. REVIEWER'S DISCUSSION:

1. MATERNAL TOXICITY: No maternal toxicity was detected in this study. The maternal adrenal and liver weight increases seen in the first dermal developmental toxicity study were not seen in this study because they were not determined. Therefore, the NOEL is > 144-169 mg/kg/day (HDT), analytically determined dose level.

2. DEVELOPMENTAL TOXICITY:

a. Deaths/Resorptions: None

b. Altered Growth: Although, the AGD was only nominally decreased, the highest dose level should not be considered the NOEL. The NOEL should remain the 60 mg/kg/day based on the NOEL seen for AGD and AGD index in the first dermal developmental toxicity study (MRID# 41413001). The highest dose level of 144 to 169 mg/kg/day should be considered a threshold level.

c. Developmental Variations: Not studied

d. Malformations: No dose related effects.

C. STUDY DEFICIENCIES: The target doses of 200 mg/kg/day were about 28% lower or about 144 to 169 mg/kg/day. Consequently, the highest dose level may not have been sufficiently high to cause the statistically significantly decreased AGD and AGD index seen in the first study at 180 and 360 mg/kg/day (MRID# 41413001, HED Doc.# 007576 and 008556).

These deficiencies have no impact, since the registrant agrees that the NOEL for dermal developmental toxicity is 60 mg/kg/day from the first dermal toxicity study not the 144-169 mg/kg/day from the current study.

3.9. DETERMINATION OF THE SERUM CONCENTRATION OF REG. NO. 83 258 AND THE METABOLITES M_1 AND M_2

3.9.1. Preparation of samples

An aliquot of the serum samples was mixed with about two aliquots of the mobile phase A in a microtest tube. This mixture was centrifuge for 6 minutes and the deproteinised supernatant was subsequently analysed by HPLC for Reg. No. 83 258, metabolites M_1 and M_2 (see also Fig. 3.9.1.1.).

Fig. 3.9.1.1.:
Degradation pathway of Reg. No. 83 258