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Item 5(a) of the provisional agenda*

INCLUSION OF CHEMICALS IN THE INTERIM PRIOR INFORMED CONSENT PROCEDURE - SUPPORTING DOCUMENTATION

Vinclozolin

Note from the Secretariat

1. Annexed to this note is the documentation provided by Jordan in support of their notification of final regulatory action on vinclozolin.

* UNEP/FAO/PIC/ICRC.5/1

List of Documentation Annexed to UNEP/FAO/PIC/ICRC5/12/Add.1

Supporting documentation on vinclozolin from Jordan:

Focused summary – vinclozolin
Correspondence from BASF, 1995
JMPR report 1995 – Vinclozolin
Excerpt of the Pesticide Manual, 10th edition - vinclozolin

This section should provide evidence that a risk evaluation was carried out under the prevailing conditions of the notifying country. It should confirm that criteria Annex 11 (b) are met. May include;

(d) Key finding of a national risk evaluation;

- high toxicity to humans, animals and birds;
- long residual effects assisting the environmental pollution
- Improper use by farmers (as it used in vegetables during fruit picking) resulting in poisoning cases.

(Minutes of the meeting did not clearly indicate a national data was generated, there is indication that there was some exposure information but no data available in the meeting documents).

(e) Key data reviews consulted and a brief description;

- pesticide manual-WHO

(f) Reference to national studies, e.g. toxicological and ecotoxicological studies;

No national study was carried out.

(g) Summary of actual (or potential) human exposure and or environmental fate.

Improper use by farmers (as it is used in vegetables during fruit picking) resulting in poisoning cases (source: minutes of the meeting, but no detailed data available).

111\ RISK REDUCTION AND RELEVANCE TO OTHER STATES

This section should provide evidence that the control action is of relevance to other states. Could include information on the followings;

(d) Estimation of quantities of chemicals used or imported/exported at the time of the regulatory action and if possible information on ongoing trade;

The Hashemite Kingdom of Jordan has no information available on quantities imported or used in Jordan as well as it has no information on ongoing trade.

(e) Relevance to other states, i.e. those with similar conditions of use;

The Hashemite Kingdom of Jordan has no information.

(f) Comments on the typical use of the chemical within the notifying country, with comments on possible misuse (if appropriate).

The product was registered to be used as insecticide and acaricide. Farmers may improperly use it in vegetables during fruit picking which result in poisoning cases (source; minutes of the meeting, no further data available).

Focused Summary- Vinclozolin

1\ INTRODUCTION:

This section should provide a brief statement / summary of the final regulatory actions and the reasons for the action taken (e.g. occupational health concerns, environmental concerns). Could include:

(e) The events that led to the final regulatory action;

The manufacturer (BASF) had submitted information indicating hazards of this product to human health. Further the company owning registration did not asked for renewal (No written documents shown to the ICRC expert preparing this summary. The available source was interview with Lina Alhamoud).

(f) Significance of the regulatory action, e.g. one use or many uses, level or degree of exposure;

The action would result in complete risk reduction especially for pregnant women as the product can cause harm to unborn child.

© An overview of the regulatory system of the notifying country if relevant;

Pesticides were used to be regulated by the law of Agriculture No. 20 for the year 1973, through a multi-stake holder committee called the Agricultural Pesticides Committee. Recently the law was amended to the Interim Law of Agriculture No. 44 for the year 2002. According to this law a national multi-stake holder committee called Pesticides Registration Committee is formed and responsible for registration, re-registration and cancellation of registration of pesticides within the Hashemite Kingdom of Jordan. The pesticide division within the ministry of agriculture is responsible for approval of label while the provinces had the authority of granting license for retailers as well as inspection of any miss-use or off law activities.

(f) Scope of the regulatory action-precise description of the chemicals subject to the regulatory action;

It is prohibited to place on the market or use plant products containing vinclozolin.

11\ RISK EVALUATION;

This section should provide evidence that a risk evaluation was carried out under the prevailing conditions of the notifying country. It should confirm that criteria Annex 11 (b) are met. May include;

(h) Key finding of a national risk evaluation;

Pregnant women and unborn child may be at risk (only hazard information, No national data).

(i) Key data reviews consulted and a brief description;

Information submitted by the manufacturer (BASF), (Not available to the ICRC expert who helped in preparing this summary).

(j) Reference to national studies, e.g. toxicological and ecotoxicological studies;

There was no national study carried out.

(k) Summary of actual (or potential) human exposure and or environmental fate.

Pregnant women and unborn child may be at risk as product may cause harm to unborn child. (Only hazard information, No national data reflecting actual exposure)

111\ RISK REDUCTION AND RELEVANCE TO OTHER STATES

This section should provide evidence that the control action is of relevance to other states. Could include information on the followings;

(g) Estimation of quantities of chemicals used or imported/exported at the time of the regulatory action and if possible information on ongoing trade;

In 1992 Jordan had imported and used 962 Kg of this product. The Hashemite Kingdom of Jordan has no information on ongoing trade.

(h) Relevance to other states, i.e. those with similar conditions of use;

The Hashemite Kingdom of Jordan has no information

(i) Comments on the typical use of the chemical within the notifying country, with comments on possible misuse (if appropriate).
The product was registered to be used as fungicide.

Focused Summary- Mevinphos

1\ INTRODUCTION:
This section should provide a brief statement / summary of the final regulatory actions and the reasons for the action taken (e.g. occupational health concerns, environmental concerns). Could include:

(g) The events that led to the final regulatory action
The insecticide Mevanate 24 % SC was presented for registrationby the Arab Company for Manufacture of pesticide (intended to be produced locally), the active ingredient is mevinphos. The control action was taken in the session No. 331, dated 9/8/1994.

(h) Significance of the regulatory action, e.g. one use or many uses, level or degree of exposure;
The banning of mevinphos would reduce the hazards to human health, low residues on crops and more healthy food for consumers and workers (source; notification form).

© An overview of the regulatory system of the notifying country if relevant;
Pesticides were used to be regulated by the law of Agriculture No. 20 for the year 1973, through a multi-stake holder committee called the Agricultural Pesticides Committee. Recently the law was amended to the Interim Law of Agriculture No. 44 for the year 2002. According to this law a national multi-stake holder committee called Pesticides Registration Committee is formed and responsible for registration, re-registration and cancellation of registration of pesticides within the Hashemite Kingdom of Jordan. The pesticide division within the ministry of agriculture is responsible for approval of label while the provinces had the authority of granting license for retailers as well as inspection of any miss-use or off law activities.

(g) Scope of the regulatory action-precise description of the chemicals subject to the regulatory action;
It is prohibited to place on the market or use plant products containing mevinphos. The decision at that time was the refusal of registration for local production of the formulation Mevanate 24% SC. This decision was interpreted to include all formulations containing mevinphos.

11\ RISK EVALUATION;
This section should provide evidence that a risk evaluation was carried out under the prevailing conditions of the notifying country. It should confirm that criteria Annex 11 (b) are met. May include;

(l) Key finding of a national risk evaluation;
- High toxicity of the product.

(Minutes of the meeting did not clearly indicate a national exposure data was generated or considered in making a national risk evaluation).

Presse-Information

BASF

September 1, 1995
P 238 e
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Vinclozolin
BASF takes precautionary safety measures for users

In order to increase protection of users of the crop-protection agent vinclozolin, BASF has taken a number of further safety precautions:

- Attention will be drawn prominently on the packaging to the need for strict observance of the prescribed safety precautions and the resultant protective measures.

- With effect from September 1, 1995, only WP (powder) formulations in water-soluble film bags will be supplied; dusts will no longer be produced. Other vinclozolin formulations, such as WG (water-dispersible granules) and SC (suspension concentrates), are not affected.

- After consultation with the approval authorities, the recommended application rate for vinclozolin-containing products will be limited to a maximum of 1 kg of active ingredient/ha per treatment.

- Vinclozolin-containing products are recommended for use in grapes, oilseed rape and beans. Other applications will be agreed separately with the national authorities.

In taking these measures, BASF is reacting to additional studies it has carried out in the course of regular re-registration proceedings. The aim was to obtain even more information on vinclozolin and its mode of action.

As part of these studies, animal experiments showed that pregnant rats fed with high doses of vinclozolin produced deformed offspring. Further studies showed how these deformations occurred.

Knowledge of the mode of action enabled a no observed effect level to be determined in the animal experiments. This value was scaled up to humans, with incorporation of a 250x safety factor, as is usual in scientific practice. A risk assessment was then obtained from a comparison with the amounts of active ingredient that a person may unavoidably be exposed to.

Based on these investigations it can be stated today:

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When vinclozolin is handled as prescribed, there are no health risks to producers and users.

The German authority responsible for health matters, the BgVV, with whom BASF has raised the question of labeling and classification, has decided that vinclozolin-containing products should, by way of precaution, be labeled with the following additional risk phrases:

R 40 - Possible risk of irreversible effects
R 62 - Possible risk of impaired fertility
R 63 - Possible risk of harm to the unborn child

Vinclozolin is an active ingredient from the dicarboximide group and is marketed by BASF as a crop protection agent for use against certain fungal diseases.

Vinclozolin-containing products have been approved by national authorities since 1976 - in Germany by the BBA, in the USA by the EPA and in Japan by the MAFF.

As for all other approved crop protection agents, approval was granted after scientific evaluation had shown that, when used as prescribed, there are no health risks for producers or users.

In Germany, the BgVV confirmed on August 11, 1995, that 'the user is sufficiently protected if the active ingredient or formulations thereof are used correctly and as prescribed'.

BASF wishes expressly to point out that consumers are and always have been protected at all times. There is no health risk in consuming products treated with vinclozolin.

VINCLOZOLIN

First draft prepared by
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Explanation

Vinclozolin was previously evaluated by the Joint Meeting in 1986 and 1988 (Annex I, references 47 and 53). In 1986, although the data were considered to be incomplete, sufficient information was provided to estimate a temporary ADI. It was noted that a plant metabolite (Metabolite T) had been identified that was not found in rats. It was concluded that vinclozolin had a low order of acute toxicity, that studies of carcinogenicity demonstrated no potential for oncogenicity, and that it had no specific mutagenic, teratogenic, or developmental effects. A temporary ADI of 0-0.04 mg/kg bw was allocated on the basis

of an NOAEL of 7 mg/kg bw per day for histological changes in spleen, prostate, and bone marrow in a six-month study in dogs and a 200-fold safety factor. In 1988, the Meeting evaluated limited data on the acute toxicity and mutagenicity of metabolite T and noted that the chemical was a transient residue in only two commodities. An ADI of 0-0.07 mg/kg bw was allocated using the same NOAEL as that used in 1986 and a safety factor of 100.

The compound was reviewed at the present Meeting within the CCPR periodic review programme. This monograph summarizes new data and that not previously reviewed and relevant data from previous monographs on this pesticide.

Evaluation for acceptable daily intake

1. Biochemical aspects

(a) Absorption, distribution, and excretion

Five male rats (strain unspecified) were given five daily doses of 40 mg/kg bw [U-^{14}C-phenyl]-vinclozolin by gavage. Excreta were collected daily and frozen, and 4 h after the last treatment the rats were sacrificed and tissues were collected and frozen. Urine was measured directly for radiolabel, whereas samples of faeces, blood, and tissues were combusted and levels of ^{14}C-carbon dioxide were determined. Data were expressed as the concentration of vinclozolin in excreta, blood, and tissues. The highest average concentration was found in faeces, followed by urine, kidney, liver, fat, muscle, and blood. The levels in urine and faeces appeared to reach a plateau by the second day of treatment (Otto *et al.,* 1977)

[U-^{14}C-phenyl]-Vinclozolin was administered orally for seven days at a dose of 40 mg/kg bw per day. Six days after the last dose, a mean of 47% of the total administered dose had been eliminated in urine and 54% in faeces. No radiolabel was detected in carcasses at this time, and none was detected in expired air. Cannulation of bile ducts after a single oral dose resulted in excretion of 65% of the administered radiolabel in the bile and only 19% in urine and 15% in faeces. Peak plasma levels were detected after about 1 h; the plasma

half-life was 20 h. As treatment continued, the baseline plasma levels tended to increase. After seven doses, the highest levels of radiolabel were detected in the liver, kidneys, gastrointestinal tract, fat, adrenals, and ovaries. By 196 h after the last dose, the levels in tissues were no different from those in plasma. These findings were confirmed by whole-body autoradiography (Chasseaud *et al.,* 1976)

The biokinetics of [U-^{14}C-phenyl]-vinclozolin was studied in male and female Wistar rats. For studies of excretion balance and plasma kinetics, five animals of each sex per test group were used; for studies of extended tissue distribution and accumulation, biliary excretion, and plasma kinetics after dietary administration of ^{14}C-vinclozolin, three animals per group were used. In a pilot study, no detectable radiolabel was excreted in expired air. There were no apparent sex differences in the routes of excretion of radiolabel. For five days after a single oral dose at a nominal level of 10 mg/kg bw (with or without a 14-day pretreatment with non-radiolabelled vinclozolin), the mean urinary excretion of radiolabel was 52-55% of the dose and the mean faecal excretion was 34-46%; 0.7-1.4% was retained. For five days after a single oral dose of ^{14}C-vinclozolin at a nominal level of 100 mg/kg bw, males excreted mean levels of 48% of the dose in urine and 49% in faeces and females excreted 54% in urine and 40% in faeces; males retained 0.6% of the dose and females 1.1%. For five days after a single intravenous dose of ^{14}C-vinclozolin at a nominal level of 1 mg/kg bw, the overall mean levels (for animals of each sex) excreted were 72% in urine and 23% in faeces. In rats with cannulated bile ducts, males excreted a mean of 73% of the radiolabel in bile and females 64% up to 48 h after a single oral dose of 10 mg/kg bw ^{14}C-vinclozolin. After a single oral dose of 100 mg/kg bw, means of 62% in males and 39% in females were excreted in bile. These results indicate that pronounced enterohepatic recirculation of radiolabel occurs in intact rats. After single oral doses of ^{14}C-vinclozolin at nominal levels of 10, 100, or 200 mg/kg bw, the time taken to reach peak plasma concentrations of radiolabel (C_{max}) tended to increase with increasing dose. Once peak levels had been reached, the concentrations declined in an apparently biphasic manner, with overall mean half-lives of 23 h for male rats

and 36 h for females. The C_{max} values and the areas under the plasma radiolabel concentration-time curves (AUC) were apparently linearly related to dose at the higher levels, whereas they were proportionately higher at the lowest dose, probably due to a greater extent of absorption. During ingestion of diet containing ^{14}C-vinclozolin at 5000 ppm over 24 h (equivalent to about 45 mg/kg bw), the plasma radiolabel concentrations increased over the initial 12 h, in accordance with a constant (zero-order) absorption model. After the animals were withdrawn from treated diet, the concentrations declined, with a mean half-life of about 40 h. The systemic availability of radiolabel appeared to be equivalent after administration of ^{14}C-vinclozolin by gavage or in the diet. After oral doses of ^{14}C-vinclozolin, radiolabel was widely distributed in tissues. In general, the tissue concentrations were higher in female rats than in males. After a single dose of 10 mg/kg bw, peak tissue concentrations occurred at 2 h in males and 6 h in females, the highest levels being found in liver, kidneys, fat, adrenals, and Harderian gland. The concentrations in all tissues declined in a generally linear manner with time. Five days after a single dose of 100 mg/kg bw of ^{14}C-vinclozolin, the tissue concentrations of radiolabel were highest in liver, kidneys, and female fat. With oral administration of ^{14}C-vinclozolin once daily for seven days at 10 mg/kg bw, the highest concentrations occurred mainly 6 h after the final dose, and the highest mean concentrations were again present in liver, kidneys, fat, adrenals, and Harderian gland. The concentrations in all tissues declined in a linear fashion (Hawkins *et al.,* 1990a).

In order to determine the tissue distribution in female Wistar rats of ^{14}C-vinclozolin administered orally once daily for seven days at a dose of 100 mg/kg bw, duplicate animals were studied by whole-body autoradiography 2, 6, 24, 69, and 168 h after the final administration. Radiolabel was absorbed from the gastrointestinal tract and widely distributed. After 2 h, high tissue concentrations were found in the organs involved in excretion and metabolism (gastrointestinal tract, bladder, liver, and kidneys) and in fat, adrenals, and glands in the region of the eye (intra- and exorbidal and especially the Harderian gland). After 168 h, radiolabel was detected at low levels only in the nasal mucosa, liver, kidneys, and intestinal contents. In comparison with the results of whole-body

autoradiography after the single administration in the previous study, the distribution of radiolabel was comparable, with the possible exception that more radioactivity was detected in the vicinity of the eye (Hawkins *et al.,* 1991a).

The absorption, distribution, and excretion of radiolabel were examined in groups of 24 male Wistar rats after dermal administration of single doses of 0.002, 0.02, 0.2, or 2 mg/cm^2 of [U-^{14}C-phenyl]-vinclozolin, equivalent to 0.13, 1.3, 13, and 130 mg/kg bw. The doses were applied for 10 h under a semi-occlusive dressing, and animals were sacrificed 0.5, 1, 2, 4, 10, and 72 h after the start of treatment. Absorption decreased as a percentage of increasing dose but increased with longer duration of exposure. Absorbed radiolabel was excreted in the urine and faeces, with means of 17, 12, 2, and 0.4% in urine and 8, 5, 1, and 0.2% in faeces over 72 h at the four doses, respectively. At sacrifice or 10 h after the start of treatment, the treated skin was washed with water. Unabsorbed radioactivity amounted to 53-99% of the dose. Treated skin of animals sacrificed up to 10 h contained 4-23% of the dose and that of animals sacrificed at 72 h contained 0.6-4%. At all doses, the liver contained the highest levels of radiolabel, followed by kidneys, adrenals, plasma, brain, blood, and testes (Hawkins *et al.,* 1991b).

The percutaneous absorption through rat and human epidermis of [U-^{14}C-phenyl]-vinclozolin was assessed *in vitro* in flow-through diffusion cells. The test substance was applied over 24 h at 2 or 200 µg/cm^2. Cumulative absorption after 8 h was 1.2% through human skin and 20% through rat skin after the high dose and 16% through human skin and 69% through rat skin after the low dose (Cameron & Jack, 1991).

(b) Biotransformation

Samples of urine and faeces were analysed to determine the identities of metabolites. Vinclozolin was metabolized extensively, as no parent compound was detected in the urine and 8-40% was detected in the faeces. *N*-(3,5-Dichlorophenyl)-2-methyl-2,3,4-trihydroxy-butanoic acid amide was the major metabolite, accounting for 42% of the urinary radiolabel and 60-90% of that in faeces. It was excreted

as either a glucuronide or sulfate conjugate in urine and in free form in the faeces. This metabolite was also the major species found in blood, kidneys, and liver. Other metabolites resulting from further degradation of this metabolite were formed in insignificant amounts (Otto *et al.,* 1977).

The metabolism of [U-^{14}C-phenyl]-vinclozolin was investigated after oral administration of single doses of 10 or 100 mg/kg bw to intact and bile duct-cannulated animals, administration of 10 mg/kg bw for seven days, or a 14-day pretreatment followed by a single dose of 10 mg/kg bw. Further groups were given an intravenous injection of 10 mg/kg bw or a single dose of 200 mg/kg bw and a 5000-ppm dietary concentration for 6 h. Vinclozolin was extensively metabolized after administration by either route. At least 15 phase-I and phase-II metabolites of vinclozolin were excreted in urine, and some were also excreted in bile and faeces. Nine of the more important urinary metabolites were characterized by mass spectrometry, enzyme deconjugation, and comparison with reference standards. These metabolites, together with unchanged vinclozolin, accounted for about 80% of the urinary and faecal radiolabel and for more than 60% of single oral doses of 10 or 100 mg/kg bw of ^{14}C-vinclozolin. The structures of these metabolites show that several competing pathways of biotransformation of vinclozolin exist in the rat (Figure 1). The phase-I pathways include: (1) hydrolytic opening of the oxazolidine ring system, involving cleavage of the 2-, 3-, or 3,4-nitrogen-carbon bonds, the former being followed by decarboxylation; (2) addition of two hydroxyl groups to the vinyl group, presumably via an epoxide intermediate; and (3) aromatic hydroxylation. The polyhydroxy compounds resulting from pathways (1) and (2) were extensively conjugated with glucuronic acid, while phenolic metabolites appeared to be conjugated with sulfate. The major phase-I metabolite of vinclozolin in the rat (R8) was the product of cleavage of the 2,3-bond in the oxazolidine ring and dihydroxylation of the vinyl group. This metabolite was excreted mainly in urine as the glucuronide conjugate, although the free aglycone was also detected. Owing to deconjugation by intestinal microflora, the free aglycone was a major component in faeces (Hawkins *et al.,* 1990b).

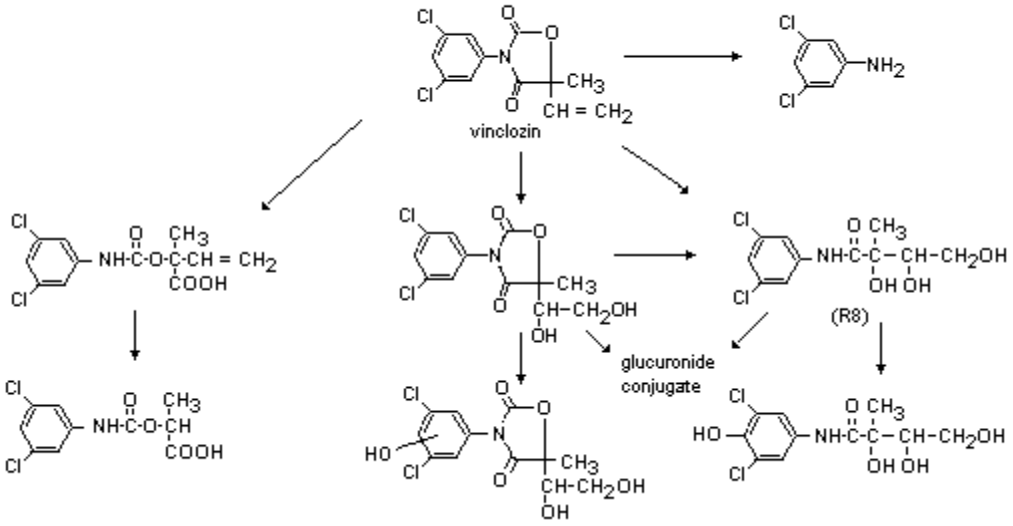
A proposed metabolic pathway for vinclozolin is shown in Figure

1.

(c) Effects on enzymes and other biochemical parameters

The acute pharmacological effects of vinclozolin were investigated in a series of studies *in vitro* and *in vivo* designed to assess effects on the central nervous system, respiratory and circulatory systems, autonomic nervous system, skeletal muscle innervation, and blood. Vinclozolin increased the mean sleeping time induced by hexobarbitone in mice and delayed pentetrazole- and strychnine-induced convulsions in mice. It had no effect on body temperature in rats or rabbits, on heart rate, respiratory rate, or blood pressure in rabbits, or on electrically stimulated muscle response in rats. Intestinal motility (charcoal propulsion in mice) was also not affected. Blood coagulation parameters were not affected in rats, but moderate haemolysis of an erythrocyte suspension was seen (Block *et al.*, 1987).

Figure 1. Proposed metabolic pathway of vinclozolin in rats



After oral administration of 2000 or 5000 mg/kg bw vinclozolin to male Sprague-Dawley rats, the effects on the cortical electroence-phalogram were examined. At both doses, sleeping stages were prolonged and the number and duration of rapid eye movement phases were slightly reduced; however, these alterations were not pronounced and suggest only a moderate sedative effect (Kretzschmar *et al.*, 1987).

2. Toxicological studies

(a) Acute toxicity

The results of studies of the acute toxicity of vinclozolin are summarized in Table 1. The clinical signs of toxicity after treatment with vinclozolin were generally nonspecific, and no consistent, treatment-related effects were seen at autopsy.

The LD_{50} of metabolite T,3,5-dichlorophenylcarbamoyl-2-propionic acid, was reported to be 2740 mg/kg bw in rats and > 2000 mg/kg bw in mice. Clinical signs of reaction to treatment included dyspnoea, staggering, piloerection, and poor general state (Kirsch, 1986a,b).

Table 1. Acute toxicity of vinclozolin

Species	Route	LD_{50} or LC_{50} (mg/kg bw or mg/litre air)	Purity (%)	Reference
Mouse	Oral	> 15 000	92.8	Shirasu et al. (1978a)
Mouse	Intraperitoneal	1570-1640	92.8	Shirasu et al. (1978a)
Mouse	Subcutaneous	> 15 000	92.8	Shirasu et al. (1978a)
Rat	Oral	> 15 000	92.8	Shirasu et al. (1978b)
Rat	Intraperitoneal	4220-8300	92.8	Shirasu et al. (1978a)
Rat	Dermal	> 5000	92.8	Shirasu et al. (1978b)
Rat	Inhalation	> 29.1	NR	Leuschner (1979)
Guinea-pig	Oral	8000	90-97	Hofmann (1973a)
Guinea-pig	Intraperitoneal	3000	90-97	Hofmann (1973b)
Rabbit	Oral	> 5620	98.1	Gelbke & Kirsch (1981)
Dog	Oral	> 10 000	97	Gelbke & Kirsch (1979)

NR, not reported

(b) Short-term toxicity

Mice

Groups of 10 male and 10 female B6C3F1 mice were fed diets containing vinclozolin at doses of 0, 100, 1000, 2500, or 5000 ppm for three months. Food consumption and body weight were determined weekly; clinical signs were checked daily, with a weekly comprehensive clinical examination. At the end of the study, clinicochemical and haematological examinations were performed, and all animals were

assessed grossly and histopathologically. Reduced body-weight gain was seen in males at the high dose at the end of the study. Clinico-chemical parameters were affected at doses ≥ 1000 ppm and haematological parameters only at the highest dose. Decreased triglyceride and cholesterol levels were seen in animals at 1000 ppm, and decreased glucose levels in males and albumin levels in females at the three higher doses. Increased alkaline phosphatase activity was seen in males and increased globulins in males and females at 2500 and 5000 ppm; alanine aminotransferase activity was increased only in males at 5000 ppm. Increases were seen in the mean corpuscular haemoglobin value in females at the two highest doses, the mean corpuscular volume in animals of each sex, haemoglobin in males, and reticulocyte counts in females at the highest dose. Absolute and relative liver weights were found to be increased at the three higher doses. Centrilobular hypertrophy of hepatocytes was seen in males at 2500 and 5000 ppm; testicular weights were increased at these doses, and multifocal hyperplasia in Leydig cells was noted at 1000 ppm or more. Absolute and relative adrenal gland weights were increased in males at 2500 and 5000 ppm, and lipogenic pigment and lipid vacuoles in the adrenals were noted in animals of each sex at these doses. In females at 1000 ppm, increased lipogenic pigment was seen in the adrenals. Hyperplasia in stromal cells of the ovaries was observed at the highest dose only. No treatment-related adverse effects were seen at 100 ppm. The NOAEL was 100 ppm, equivalent to about 20 mg/kg bw per day (Schilling *et al.,* 1990a).

The B6C3F1 mouse strain has a relatively high frequency of spontaneous liver lesions, including tumours. In the three-month study with this strain, clear increases in liver weight and hepatocellular hypertrophy were observed, indicating that the liver is one of the target organs of vinclozolin. In order to assess more accurately its potential proliferative effect on mouse liver, the toxicity of vinclozolin was investigated in C57B1 mice, which have a low background incidence of spontaneous liver neoplasia. Groups of 10 male and 10 female mice were fed diets containing vinclozolin at doses of 0, 100, 1000, or 5000 ppm. Food consumption and body weight were determined weekly, and clinical signs were checked daily, with a weekly comprehensive clinical examination. At the end of the study,

clinicochemical and haematological examinations were performed. All animals were assessed macroscopically and histologically. A reduction in body-weight gain was seen in males at the high dose. Decreased triglyceride and cholesterol values were seen in animals at 1000 and 5000 ppm, and glucose was decreased only in males at the high dose. Increased alanine aminotransferase activity was seen in animals of each sex, and total protein and alkaline phosphatase were increased in males at the high dose. Haemoglobin, mean cell volume, and mean corpuscular haemoglobin were increased in animals of each sex at 5000 ppm and in females at 1000 ppm; haematocrit and leukocyte and lymphocyte counts were increased in animals of each sex and the erythrocyte count in males at the highest dose. Increased liver weights were seen at 1000 and 5000 ppm, and centrilobular hypertrophy of hepatocytes was seen at 5000 ppm. Increased adrenal weights were seen males at this dose; an increase in lipogenic pigment was noted histologically in animals of each sex at 1000 ppm and 5000 ppm and lipid vacuoles were seen at 5000 ppm. Hyperplasia and hypertrophy of the stromal cells of the ovaries were seen at the middle and high doses, and focal hyperplasia of the Leydig cells of the testis was observed at the high dose. There were no compound-related findings at 100 ppm. The NOAEL was 100 ppm, equivalent to 25 mg/kg bw per day (Schilling *et al.,* 1990b).

Rats

Groups of 16 male and 16 female Sprague-Dawley rats were fed diets containing 0,100, 300, 1000, or 2000 ppm technical-grade vinclozolin for three months, and six rats from each group were further maintained on control diets for six weeks after the end of the study. Rats were examined daily for mortality, abnormal appearance or behaviour, and food consumption. Body weights were determined weekly, when rats were palpated and the eyes examined. Blood and urine were sampled before treatment, after six and 12 weeks of treatment, at termination, and at the end of the observation period. At termination, rats were sacrificed, dissected, and examined for gross pathological changes. The absolute and relative weights of major organs were determined, and a complete set of tissues from each animal was saved for future histopathological examination. The results of microscopic

examinations of tissues were not reported, except for the eyes, which were examined in serial sections. A single death occurred, in a female at the high dose on day 42; all other rats survived to scheduled termination. No abnormalities of appearance or behaviour were noted, and the eyes were normal at all examinations. The body weights of treated rats were comparable to those of controls throughout the study. Occasional statistically significant increases in food consumption were seen in treated females, but that of males was not affected. Haematological changes consistent with decreased erythrocyte mass-decreased erythrocyte count, haematocrit, and haemoglobin and increased mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration-were seen at the six-week sampling time in

males and females fed doses $\geq$ 300 ppm; however, these changes were seen at termination only in females at 1000 and 2000 ppm. Clinical chemical parameters were not altered in a toxicologically significant manner. At necropsy, no effects of treatment on the gross appearance of tissues were apparent. Statistically significant, dose-related increases in the mean absolute and relative weights of the liver and adrenals were seen in males and females at 1000 and 2000 ppm; at 2000 ppm, increased relative weights of kidneys were seen in males and females and increased relative spleen weights in females. These effects were apparently reversible, as they were not observed in treated rats that received control diets for an additional six weeks. Microscopic examination of the eyes revealed no treatment-related abnormalities (Hofmann, 1974).

Groups of rats received vinclozolin at dietary levels of 0, 900, 1800, 3000, or 15 000 ppm for four weeks. There were no clinical signs of toxicity, and food intake and body-weight gain were reduced only in animals at the high dose. Erythrocyte parameters were reduced in all treated female rats, and urinalysis revealed reduced osmolality at the highest dose. Liver and adrenal weights were increased in all treated rats, and necropsy revealed a grey-white discolouration of the adrenals. The ascorbic acid content of the adrenals was increased in all treated animals, and the glycogen content of the liver was reduced in all treated females and in males at 15 000 ppm. As progressive, dose-related transformation of the adrenal cortex was seen in all treated rats, there was no NOAEL (Hoffman & Munk, 1975a).

Groups of 50 male and 50 female Sprague-Dawley rats received vinclozolin in the diet at levels of 0, 150, or 450 ppm for three months. Clinical behaviour, food and drinking-water consumption, and body-weight gain were not affected, and clinicochemical examinations and urinalyses revealed no substance-induced changes. Histopathological examination of the liver, adrenals, and pituitary at the end of treatment gave no reliable indication of substance-induced changes. The NOAEL was > 450 ppm, equal to 44 mg/kg bw per day for males and 40 mg/kg bw per day for females (Leuschner *et al.,* 1975).

Groups of 10 male and 10 female Wistar rats received vinclozolin in the diet at levels of 0, 300, 1000, or 3000 ppm for three months. There were no clinical signs of reaction to treatment, and no rats died before the scheduled sacrifice. Body-weight gain and food intake were unaffected by treatment, but water intake was increased in rats at 3000 ppm. Ophthalmoscopic examination revealed two cataracts, one unilateral and one bilateral, in rats receiving 3000 ppm, and other lenticular changes were seen in all groups; it was concluded that the ocular lesions were spontaneous and unrelated to treatment. Evidence of anaemia and decreased serum alkaline phosphatase activity were seen in animals at the high dose, with decreases in leukocyte counts at 1000 and 3000 ppm. Examination *post mortem* revealed white, enlarged adrenals in all treated rats. The weights of the liver, adrenal, and

testis were increased at 1000 and 3000 ppm, and histopathological examination revealed hypertrophy of the adrenal cortex, cystoid degeneration in the pituitary, Leydig-cell hyperplasia, cloudy swelling of hepatocytes, single live cell necrosis, and vacuolization of luteal cells in the ovaries at these doses. Acinar vacuolization in the pancreas was seen in all treated rats. Vinclozolin was thus toxic at all doses; the lowest dietary level of 300 ppm is equal to 22 mg/kg bw per day (Mellert *et al.,* 1993a).

In order to define a clear no-effect level, groups of 10 male and 10 female Wistar rats received vinclozolin in the diet at levels of 0

or 50 ppm for three months, and investigations similar to those in the previous study were performed. In particular, laboratory investigations and ophthalmoscopic examinations were carried out, and all animals underwent detailed pathological examination. No treatment-related changes were seen. The NOAEL was 50 ppm, equal to 4 mg/kg bw per day (Mellert *et al.,* 1993b).

Rabbits

Groups of six New Zealand white rabbits received dermal applications of vinclozolin for 8 h per day for three weeks at doses of 0, 111, 333, or 1000 mg/kg bw. There were no deaths and no clinical signs of reaction to treatment; gross pathology and histopathology revealed no evidence of dermal irritation (Leuschner *et al.,* 1977a).

Dogs

Groups of four beagle dogs of each sex were fed diets containing vinclozolin at doses of 0, 100, 300, 1000, or 2000 ppm for three months. There were no signs of toxicity and no compound-induced deaths. Repeated ophthalmological examinations revealed no effects. The body-weight gain of treated animals was not affected, and urinalysis showed no treatment-related findings. Females at 2000 ppm had a reduced haemoglobin content after four and eight weeks and a decreased erythrocyte count throughout the study. An increased platelet count was seen in female animals at 1000 and 2000 ppm. Howell-Jolly bodies were found in a differential blood count in males and females at these doses, suggesting a compensatory reaction elicited by an anaemic process. The relative liver weights and the relative and absolute adrenal weights were increased in females at the highest dose. Histopathological assessment revealed compound-induced cholestasis of the liver. In a further evaluation, there was a slight to moderate, dose-related increase in the haemosiderin content of the liver, particularly in females, at 300, 1000, and 2000 ppm. Females had a higher pigment content than males, and the spleens of females also had an increased haemosiderin content. It was assumed that the haemosiderosis in the liver was caused by increased haemolysis, in

contrast to the original interpretation that the pigment deposits in the liver were due to cholestasis. The NOAEL was 100 ppm, on the basis of the increased haemosiderin content of the liver (Hoffman & Munk, 1975b).

Pairs of one male and one female beagle dog were given technical-grade vinclozolin at doses of 0, 1000, or 2000 ppm for three months. There were no compound-induced deaths or other signs of toxicity during treatment. The animals underwent ophthalmological examinations twice a week and were additionally examined by an eye specialist towards the end of the treatment period. There was no indication of cataract formation. The NOAEL was thus > 2000 ppm (Kirsch *et al.,* 1974).

Groups of six beagle dogs of each sex received diets containing technical-grade vinclozolin at doses of 0, 100, 300, 600, or 2000 ppm for six months. Clinicochemical, haematological, and urinalyses were carried out at regular intervals; ophthalmoscopy was performed before the beginning of the study, after about three months, and towards the end of treatment. At the end of the study, all animals were examined grossly and histopathologically. No deaths occurred, food consumption and body-weight gain were unchanged in comparison with the control group, and there were no clinical signs of toxicity. Males at the highest dose had pronounced, and females slight, haemolytic anaemia. Increased incidences of Howell-Jolly bodies and reticulocytes in males suggested a compensatory bone-marrow reaction to the compound-induced loss of blood cells. Slight increases in bilirubin level in males at 600 and 2000 ppm, in the haemoglobin concentration of individual erythrocytes, and in lactic dehydrogenase activity in males at 2000 ppm were further consequences of increased decomposition of erythrocytes. The considerably increased platelet counts in males at 2000 ppm and the slight increase in females at 600 and 2000 ppm are probably due to the anaemic process, since hyper-regenerative anaemia is frequently accompanied by thrombocytosis. The absolute and relative adrenal weights showed a dose-related increase in groups treated with 2000, 600, or 300 ppm, and histopathological examination showed

vacuolization of the zona fasciculata and severe lipid incorporation and birefringence of the adrenal cortex (in females) at the highest dose. Increased absolute and relative spleen weights were observed at 2000 ppm; at 600 ppm, only the absolute spleen weight was increased in females. Histopathological examination revealed dilated splenic sinuses and hyperaemia in females at both doses. Reduced relative weights of the kidney (at 2000 ppm) and pituitary (at 600 and 2000 ppm), haemosiderin deposits in the liver (at 600 and 2000 ppm), and severe prostatic atrophy (at 2000 ppm) were also found. Ophthalmological examinations revealed no compound-related findings. The NOAEL was 100 ppm, equivalent to 4.0 mg/kg bw per day (Kirsch *et al.,* 1982).

Groups of six beagle dogs of each sex were fed diets containing 0, 35, 75, 150, or 1500 ppm vinclozolin for 12 months. There were no deaths and no clinical signs of toxicity. Food consumption and body-weight gains were not significantly affected by treatment. Haematological, clinical chemical, and urinalyses were conducted before treatment and in weeks 13, 26, and 52. At the highest dose, reticulocyte counts were increased in animals of each sex, platelet counts were increased in males, the mean cell volume was increased in females, and total bilirubin concentrations were increased in animals of each sex. Adrenal weights were increased at 1500 ppm and slightly increased at 150 ppm in animals of each sex; the adrenals were enlarged in all males and in five females at 1500 ppm, and the mean width of the adrenal cortex was increased in animals of each sex at 1500 ppm and, to a lesser degree, in females at lower doses. No dose-response relationship was identified. Progressive transformation and increased lipid content were seen in the adrenals of all males and five females at 1500 ppm. Testicular weights were increased in a dose-related fashion; at 1500 ppm, the weights were markedly increased, and at 150 ppm the relative weights were significantly increased. At 1500 ppm, diffuse hyperplasia of the Leydig cells was observed in five males. The prostates of one male at 150 ppm and two at 1500 ppm appeared smaller macroscopically. Histopathological examination revealed that the prostates of two males at 150 ppm were slightly or moderately atrophied and those of five males at 1500 ppm were slightly (one) or severely (four) atrophied; similar but minimal effects were seen in one male at 0, one at 35, and one at 75 ppm.

Liver weights were increased in animals at doses up to 150 ppm and were markedly increased in males at 1500 ppm. Increased haemosiderin deposition was observed in the livers of males at 1500 ppm and of females at 150 and 1500 ppm. Spleen and thyroid weights were increased in males at 1500 ppm, and spleen weights were also slightly increased in females at 1500 ppm. The NOAEL was 75 ppm, equal to 2.4 mg/kg bw per day, on the basis of pathological effects at 150 and 1.500 ppm (Hellwig *et al.,* 1987).

(c) Long-term toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female NMRI mice were fed diets containing 0, 162, 486, 1460, or 4370 ppm vinclozolin for 112 weeks. The results of analyses of the diets were not reported. There were no clinical signs of reaction to treatment and no effect on food intake. The weight gain of males at 1460 or 4370 ppm was reduced, and the survival of males at the highest dose was adversely affected; no such changes were seen in females. The weights of the liver and testis were increased at the highest dose, and liver weight was increased in females at 1460 ppm. Histopathological examination revealed no treatment-related changes, and no tumours were seen (Leuschner *et al.,* 1977b).

Groups of 100 male and 100 female control C57Bl/6/JICO mice and 60 male and 60 female treated animals received vinclozolin in the diet at levels of 0, 15, 150, 3000, or 8000 ppm for 18 months. Ten animals of each sex were taken from each group for interim sacrifice after 12 months of treatment. There were no clinical signs. The mortality rates of males and females at the highest dose were greater than those of controls, and weight gain and food intake were reduced in animals at 3000 and 8000 ppm. Examination of blood smears revealed an increased polymorphonuclear neutrophil count and decreased lymphocyte count in animals of each sex at 8000 ppm. Pathological examinations revealed similar findings at the interim and terminal kills. Increased liver and adrenal weights were seen in animals at 3000 and 8000 ppm, with

smaller epididymides, seminal vesicles, and prostate. Focal necrosis, bile-duct proliferation, and pigment deposition were seen in the livers of animals at 3000 or 8000 ppm. At 8000 ppm, these changes were accompanied by diffuse hepatocyte hypertrophy, decreased lipid storage, and increased focal fatty infiltration in the liver, hepatic single-cell necrosis, an increased prevalence of biliary cysts in the livers of males, focal cellular alterations and focal hyperplasia of the livers of males and females, hepatocellular carcinomas in three males and 22 females, and hepatocellular adenomas in three females. In males at 3000 and 8000 ppm, diffuse Leydig-cell hyperplasia of the testis was seen, with atrophy of the seminal vesicles and coagulation glands. Females at these doses had atrophic uteri, accompanied in animals at the highest dose by diffuse stromal hyperplasia, an increased incidence of pigmented interstitial cells in the ovaries, and loss of ovarian follicles. The adrenal cortexes of animals at 3000 or 8000 ppm had increased lipogenic pigment in the cortico-medullary region and lipidosis. In addition, in animals at 8000 ppm, foam cells, eosinophilic crystals, and pneumonitis were seen in the lungs of males and erosions or ulcers in the glandular stomachs of males and females.

Vinclozolin thus caused hepatocellular carcinomas at a dose of 8000 ppm, equal to 1300 mg/kg bw per day, a dose associated with clear toxicity; there were no treatment-related tumours at 3000 ppm, equal to 495 mg/kg bw per day, although evidence of toxicity was seen. The NOAEL was 150 ppm, equal to 24 mg/kg bw per day (Mellert *et al.*, 1994a).

Rats

Groups of 50 male and 50 female Sprague-Dawley rats were fed diets containing 0, 162, 486, 1460, or 4370 ppm vinclozolin for 130 weeks; the study was terminated when survival in the control group reached 70%. The results of analyses of the diets were not reported.

There were no clinical signs of reaction to treatment. Dose-related reductions in food intake and weight gain were seen in animals at 1460 and 4370 ppm, but their survival was better than that of controls. Clinical chemical and histological investigations showed no reaction

to treatment. Interpretation of the data on organ weights was hampered by disparities in body weight between groups. No tumours were observed (Leuschner *et al.*, 1977c).

Groups of 20 male and 20 female Wistar rats received vinclozolin in the diet at levels of 0, 150, 500, 1500, or 4500 ppm for 24 months. The only clinical signs of reaction to treatment were an increased incidence of palpable, enlarged testes in all treated males and cataracts in all treated rats. Although the mortality rate of females at the high dose was higher than that in concurrent controls, the increase was marginal and may have been unrelated to treatment. Weight gain and food intake were adversely affected in animals at 1500 and 4500 ppm. Water intake was increased in males at 4500 ppm and decreased in females at this dose and in males and females at 1500 ppm. Ophthalmoscopic examination revealed a treatment-related incidence of cataracts. Bilateral cataracts were present in all animals at 1500 and 4500 ppm that survived to termination, and cataracts were also seen at 500 ppm. The ophthalmoscopic changes at 150 ppm were confined to lenticular degeneration and calcification in a few animals. Animals at 1500 and 4500 ppm showed evidence of anaemia, decreased serum alanine aminotransferase and alkaline phosphatase activities, and increased creatinine, total protein, cholesterol, and gamma-glutamyl transferase activity. Increased liver and adrenal weights were seen in animals at 4500 ppm and increased testicular weights in all treated males. Leydig-cell tumours were seen in almost all animals treated with 500, 1500, or 4500 ppm and were increased in incidence in rats at 150 ppm in comparison with controls. Focal hyperplasia and cystic ducts were seen in the rete testis of rats at 4500 ppm, and two males at this dose but no controls had rete testicular adenomas. Cystic ducts in the rete testis were also seen in animals at 1500 ppm. Atrophy of the seminal vesicles, coagulating gland, and epididymides were seen in almost all animals at 1500 and 4500 ppm, similar, less marked effects being seen in animals at 150 and 500 ppm. Dose-related reduced secretion and increased fibrosis in the prostate were also seen in all treated males. Benign stromal tumours of the sex cord in the ovaries were seen in 10 rats treated with 4500 ppm, four at 1500 ppm, and two at 500 ppm; none were seen in

animals at 150 ppm or in controls. Five adenomas and one metastatic carcinoma of the adrenal cortex were seen in females at 4500 ppm and one adenocarcinoma of the adrenal cortex in a female at 1500 ppm; no adrenal tumours were seen at 150 or 500 ppm or in controls. A dose-related incidence of lipidosis in the adrenal cortex was seen in all treated animals. Hepatocellular carcinomas occurred in nine male rats at 4500 ppm, with none in controls. Although hepatic tumours were found only in animals at the high dose, hepatic necrosis, hypertrophy, and eosinophilic foci were seen in a dose-related fashion at doses down to 150 ppm; the only changes in animals at the low dose were eosinophilic foci in one female. Vacuolation of the pancreatic exocrine cells was seen in all treated groups. Treatment-related changes were also seen in the pituitary in animals at 1500 and 4500 ppm, consisting of a reduction in focal hyperplasia and an increase in diffuse hyperplasia in males and a reduction in the incidence of pituitary adenomas in females. Vinclozolin was toxic at all doses tested. The lowest dietary level of 150 ppm was equal to 8 mg/kg bw per day (Mellert *et al.,* 1994b).

In order to define a clear no-effect level, groups of 20 male and 20 female Wistar rats received vinclozolin in the diet at levels of 0, 25, or 50 ppm for 24 months, with investigations similar to those performed in the previous study. In particular, laboratory investigations and ophthalmoscopic examinations were carried out every three months, and all animals underwent detailed pathological examination. No treatment-related changes were seen. The NOAEL was 50 ppm, equal to 2.8 mg/kg bw per day (Mellert *et al.,* 1993b).

Groups of 50 male and 50 female Wistar rats received vinclozolin in the diet at levels of 0, 50, 500, or 3000 ppm for 24 months. The only clinical signs of reaction to treatment were an increased incidence of palpable, enlarged testes in males at 500 and 3000 ppm and cataracts in all treated rats. Mortality was not affected. Weight gain and food intake were decreased in animals at 3000 ppm. Clinical examination showed an increased incidence of cataracts in animals at 500 and 3000 ppm, but ophthalmoscopy of control animals and those at the low dose revealed an increased incidence of lenticular degeneration and one case of lenticular calcification in animals at 50

ppm. Animals at 3000 ppm had increased liver, adrenal, and testicular weights, and histopathological examination revealed Leydig-cell tumours in almost all males at 500 or 3000 ppm. Focal hyperplasia and cystic ducts in the rete testis were seen in males at 3000 ppm, and an adenoma in the rete testis was seen in one treated and no control males. Atrophy of the seminal vesicles and coagulating gland was seen in almost all males at 3000 ppm and in some at 500 ppm. Dose-related reduced secretion and increased fibrosis in the prostate were seen in all treated groups. Benign stromal tumours of the sex cord in the ovaries were seen in 29 rats at 3000 ppm and four controls. Two animals at the high dose had malignant thecomas of the ovary, with none in controls. Lipidosis of ovarian interstitial cells and an increased incidence of abnormal ovarian follicles were seen in all treated groups. Seven animals at the high dose had adenocarcinomas of the uterus (none in controls), and three of these had metastases. Adenomas of the adrenal gland were seen in 21 females at 3000 ppm, and one had a metastatic carcinoma of the adrenal cortex; there was no increase in the incidence of adrenal tumours in animals at 50 or 500 ppm. A dose-related incidence of lipidosis and focal hyperplasia in the adrenal cortex was seen in all treated groups, and hepatic hypertrophy and eosinophilic foci were seen in a dose-related fashion at doses down to 50 ppm. Vacuolation of the pancreatic exocrine cells was seen in rats at 500 or 3000 ppm. At 3000 ppm, males had an increased incidence of focal hyperplasia in the pituitary, and females had a reduced incidence of pituitary adenomas. Thus, increased incidences of tumours were seen in animals of each sex at 3000 ppm and in males at 500 ppm. The NOAEL was 50 ppm, equal to 2.7 mg/kg bw per day, for tumour formation and < 50 ppm for overall toxicity (Mellert *et al.,* 1994d).

(d) Reproductive toxicity

In a three-generation study, groups of 20 Sprague-Dawley rats of each sex were fed diets containing vinclozolin at 0, 162, 486, or 1458 ppm. Two litters were produced per generation, and the second litters were used as parental animals (F_0, F_1, and F_2). The period of treatment before mating was about eight weeks for F_0 males, 16 weeks for F_0 females, 15 weeks for F_1 and F_2 males, and 24 weeks for F_1 and F_2 females (including lactation). No treatment-related deaths or clinical signs of toxicity were seen in

pups or parents, and there were no treatment-related effects on food consumption, body weights, litter size, malformations, birth weight of pups, sex ratio, or behaviour. In parents, there were no treatment-related effects on fertility, pregnancy rate, duration of pregnancy, lactation, or viability. Auditory acuity and ophthalmic parameters were not affected by treatment. There were no treatment-related effects on organ weights or gross or microscopic appearance. The no-effect level was thus > 1458 ppm, equivalent to about 73 mg/kg bw per day. The results are not in accordance with more recent work, but this study did not include investigations of anogenital distance in males, and diets were not analysed for vinclozolin (Leuschner, 1977).

Groups of 24 Wistar rats of each sex were fed diets containing 0, 50, 300, 1000, or 3000 ppm vinclozolin. After a pre-mating period of 10 weeks, these F_0 animals were mated twice to produce F_{1a} and F_{1b} pups. F_{1a} animals were used as F_1 parents to produce F_{2a} and F_{2b} animals. Some rats were raised to adulthood in order to observe the development of their sexual organs, resulting in an FX group of F_{1b} rats, an FY group of F_{2a} rats, and an FZ group of F_{2b} rats. The results of the study are summarized in Table 2. All F_1 males and females at 300 ppm were fertile either at the F_{2b} mating or at further matings for those animals that did not prove their fertility in at least one of the scheduled matings for the F_{2a}/F_{2b} litters. The authors considered that 'clear adverse effects on fertility were noted only at 1000 and 3000 ppm, whereas 300 and 50 ppm were without any adverse effects on male and female fertility in both parental generations.' It must be noted, however, that rats are particularly fertile, and the effects observed, particularly at 300 ppm, may indicate sub-fertility. The Meeting concluded that the dietary level of 50 ppm, equivalent to 4.5 mg/kg bw per day, is a marginal-effect level, on the basis of a possible treatment-related reduction in the fertility of F_1 males at 300 ppm, signs of delayed development at 300 ppm (reduction in numbers of pups with pinna unfolding and eye opening at the expected time), and reduced epididymal weight in F_2 animals at 50 ppm (Hellwig *et al.*, 1990a).

Groups of 25 male and 25 female Wistar rats were fed diets containing vinclozolin at doses of 0, 20, or 40 ppm. After a 70-day premating period, these F_0 parents were mated to produce F_{1a} and F_{1b} animals, and F_{1a} rats were mated to produce F_{2a} and F_{2b} animals. Randomly selected F_{1b}, F_{2a}, and F_{2b} pups were

additionally raised to adulthood, resulting in FX, FY, and FZ groups, respectively. No effect on clinical signs, weight, food intake, or reproduction was observed, and gross pathological examination and organ weight analysis also revealed no reaction to treatment. In particular, there was no treatment-related effect on epididymal weight in FX, FY, or FZ animals. The NOAEL was thus 40 ppm, equal to approximately 4 mg/kg bw per day (Hellwig *et al.,* 1994).

Vinclozolin was administered to groups of pregnant rats by gavage at doses of 0, 100, or 200 mg/kg bw per day from day 14 of gestation to postnatal day 3. Male pups in both groups of offspring displayed feminine characteristics and their reproductive capacity was adversely affected. About 25% of the treated males died during the study as a result of bladder stones, hydroureter, or hydronephrosis. Malformations noted at necropsy, when the males were approximately one year of age, included the presence of a vaginal pouch, suprainguinal ectopic scrotum or testes, cleft phallus with hypospadias, and small to absent accessory sex glands. The authors proposed that vinclozolin is an androgen receptor antagonist (Gray *et al.,* 1994a).

In a study reported only in brief summary form, pregnant rats were treated with 0, 3.12, 6.25, 12.5, 25, 50, or 100 mg/kg bw per day vinclozolin from day 14 of gestation to postnatal day 3. Fertility was adversely affected at 50 and 100 mg/kg bw per day, and subtle changes in anogenital distance were observed at all doses (Gray *et al.,* 1994b).

(e) Developmental toxicity

Mice

Female NMRI albino mice were fed diets containing vinclozolin on days 1-19 of gestation in two tests. In the first test, 24 mice received 0 and 28 received 60 000 ppm vinclozolin; in the second, groups of 30 mice received 0, 600, or 6000 ppm. All animals were killed on day 19 of gestation. Females at 6000 ppm did not gain weight during gestation and had decreased food consumption during the first six days of treatment. Females at 60 000 ppm lost weight and had decreased food consumption. One female at 6000 ppm died on day 11, and all females treated at 60 000 ppm died within the first nine days. Clinical signs of toxicity were observed only in mice at 60 000 ppm

and included ruffled coats and, before death, apathy and signs of pronounced diuresis. Gross pathology revealed emaciation, atrophy of musculature, and considerable loss of perirenal fatty tissue in

Table 2. Results of a multigeneration study in Wistar rats fed diets containing vinclozolin

Dietary level (ppm)	Generation	Finding
General toxicity		
50		--
300	F_0	increased relative liver weight, marginal signs of anaemia (females), lenticular degeneration
	F_1	As F_0, plus increased adrenal weight, Leydig-cell hyperplasia
	F_2	As F_1
1000	F_0	Reduced food intake and weight gain, anaemia (females), increased liver, adrenal, and testicular weights, lenticular degeneration, hepatic single-cell necrosis, Leydig-cell hyperplasia
	F_1	As F_0, plus vacuolation of pituitary cells, lipidosis, and hypertrophy of adrenal cells
3000	F_0	Reduced food intake and weight gain, lenticular degeneration, anaemia (females), increased liver, adrenal and testicular weights, hepatic single-cell necrosis, lipidosis and hyperplasia of adrenal cells, vacuolation of pituitary cells, Leydig-cell hyperplasia
	F_1	As F_0, pills benign Leydig-cell tumours in some animals
Effects on reproductive performance		
50		--

300	F_0	--
	F_1	Possible reduction in fertility in males (all males eventually proved fertile)

Table 2. (cont'd).

Dietary level (ppm)	Generation	Finding
1000	F_0	--
	F_1	Infertility of all males due to feminization of outer genital organs
3000	F_0	Increased total litter loss, decreased number of delivered pups, infertility of males
	F_1	Infertility, of all males (feminization), infertility of six females
Signs of developmental toxicity		
50	F_1	--
	F_2	Reduced epididymal weight (no morphological changes)
300	F_1	Slight functional reduction of prostate and coagulating gland, reduced epididymal weight
	F_2	As F_1, plus slight interstitial-cell hyperplasia in testes and ovaries, some pups with reduced morphological development (delayed eye opening and pinna unfolding)
1000	F_1	Decreased pup survival, feminization of males, reduced body-weight gain, slightly delayed pup development, reduced size and function of secondary male genital organs, atrophy of seminiferous tubules, interstitial-cell hyperplasia in testes and ovaries

Table 2. (cont'd).

Dietary level (ppm)	Generation	Finding
3000	F_1	Increased number of stillborn pups, decreased pup survival, feminization of males, reduced body-weight gain, retarded morphological development, atrophy of primary and secondary male genital organs, interstitial-cell hyperplasia in testes and ovaries

From Hellwig et al. (1990a)

animals that died. No implantation sites were detected in any female at 6000 or 60 000 ppm, and hence no fetuses were observed at these doses. Fetuses of dams at 600 ppm had no treatment-related adverse effects. The NOAEL for maternal toxicity and fetotoxicity was 600 ppm, equivalent to 90 mg/kg bw per day (Hofman & Peh, 1975a).

Rats

Groups of 25 female Wistar rats were treated orally with vinclozolin at doses of 0, 15, 50, or 150 mg/kg bw per day on days 6-19 of gestation and were killed on day 20. There were no deaths, no clinical signs of toxicity, no abortions, and no treatment-related effects on food consumption or body weight. Haematological investigations performed at day 20 revealed no treatment-related effects, and no adverse findings were noted at gross necropsy. There were no treatment-related effects on pre- or post-implantation losses, resorptions, numbers of live fetuses, or fetal or placental weights. Anogenital distances were decreased in male fetuses at 150 and, to a lesser extent, 50 mg/kg bw per day. This effect was considered to indicate the beginning of feminization of male fetuses, perhaps due to a hormonal (anti-androgenic) action on sexual differentiation. There

were no other treatment-related effects on male or female fetuses. As no maternal toxicity, embryotoxicity, or fetotoxicity was observed, the NOAEL was > 150 mg/kg bw per day. The NOAEL for teratogenicity was 15 mg/kg bw per day, on the basis of reductions in anogenital distance at 50 and 150 mg/kg bw per day (Hellwig *et al.,* 1989a).

Groups of 25 female Wistar rats were treated orally with vinclozolin at doses of 0, 50, 100, or 200 mg/kg bw per day on days 6-19 of gestation and were killed on day 20. There were no deaths, no clinical signs of toxicity, no abortions, and no treatment-related effects on food consumption or body weight. Haematological investigations performed at day 20 revealed no treatment-related findings, and no treatment-related adverse signs were noted at necropsy. There were no treatment-related effects on pre- or post-implantation losses, resorptions, numbers of live fetuses, or placental or fetal weights. The only possibly treatment-related morphological effect observed in the fetuses was a significantly increased incidence of symmetrically dumb-bell-shaped thoracic vertebral bodies at 200 mg/kg bw per day, which is indicative of retarded development. A slight, not statistically significant increase was observed at 100 mg/kg bw per day. Similar effects were observed in a follow-up study. The NOAEL for maternal toxicity was > 200 mg/kg bw per day, and that for fetotoxicity was 100 mg/kg bw per day, on the basis of a possible treatment-related increase in symmetrically dumb-bell-shaped thoracic vertebral bodies at 200 mg/kg bw per day. There was no NOAEL for teratogenicity, as the anogenital distance in fetuses was not measured (Hellwig *et al.,* 1989b).

Groups of 25 female Wistar rats were treated orally with vinclozolin at doses of 0, 200, or 400 mg/kg bw per day on days 6-19 of gestation and were killed on day 20. There were no deaths, no clinical signs of toxicity, no abortions, and no treatment-related effects on food consumption or body weight. Haematological investigations performed at day 20 revealed no treatment-related findings, and no treatment-related adverse signs were noted at gross necropsy. There were no treatment-related effects on pre- or post-implantation losses, resorptions, numbers of live fetuses, fetal or placental weights, or sex ratio. The anogenital distances of male fetuses were decreased in a dose-related fashion at both 200 and 400 mg/kg bw per day, indicating the beginning of feminization. A treatment-related increase in dilated renal pelvis and hydroureter was also observed in fetuses of animals of each sex, which were

statistically significant in fetuses at 400 mg/kg bw per day. These findings are considered to indicate a transient developmental delay.

There was also a significant increase in the number of fetuses with symmetrical dumb-bell-shaped thoracic vertebral bodies at 200 and 400 mg/kg bw per day, indicative of retarded development. At 400 mg/kg bw per day, the numbers of fetuses with accessory 14th ribs were also significantly increased. The NOAEL for maternal toxicity was > 400 mg/kg bw per day, and that for fetotoxicity was < 200 mg/kg bw per day, on the basis of the increased incidences of symmetrical dumb-bell-shaped thoracic vertebral bodies at 200 and 400 mg/kg bw per day in animals of each sex. The NOAEL for teratogenicity was < 200 mg/kg bw per day, on the basis of reductions in anogenital distance in male fetuses at 200 and 400 mg/kg bw per day (Hellwig *et al.,* 1989c).

Groups of 10 female Wistar rats were treated orally with vinclozolin at doses of 0, 600, or 1000 mg/kg bw per day on days 6-19 of gestation and were killed on day 20. There were no deaths and no abortions. Clinical signs of toxicity included unsteady gait in one female at 600 mg/kg bw per day and in seven females at 1000 mg/kg bw per day, and piloerection in two females at 1000 mg/kg bw per day. Food consumption was reduced in animals at 1000 mg/kg bw per day on days 6-13 of gestation, and the water consumption of animals at 600 and 1000 mg/kg bw per day was increased. The body-weight gain of females at 1000 mg/kg bw per day was impaired on days 8-10. Haematological investigations performed at day 20 revealed no treatment-related findings. Liver and adrenal weights were increased in a dose-related fashion. There were no treatment-related effects on pre- or post-implantation losses, resorptions, numbers of live fetuses, or placental weights. Fetal weights were decreased at 1000 mg/kg bw per day. When the sex ratio was determined by measuring the anogenital distance, 68% of fetuses at 600 and 99% at 1000 mg/kg bw per day were deemed to be female; however, internal examination showed that 39% at 600 and 52% at 1000 mg/kg bw per day were female.

The anogenital distances were decreased in treated male fetuses. At 1000 mg/kg bw per day, the incidences of symmetrical dumb-bell-shaped thoracic vertebral bodies were increased. The NOAEL for maternal toxicity was < 600 mg/kg bw per day, on the basis of increased liver

and adrenal weights and findings at necropsy at 600 and 1000 mg/kg bw per day. The NOAEL for fetotoxicity was < 600 mg/kg bw per day, on the basis of increased incidences of hydroureter at 600 and 1000 mg/kg bw per day. The NOAEL for teratogenicity was < 600 mg/kg bw per day, on the basis of reductions in anogenital distance in male fetuses at 600 and 1000 mg/kg bw per day (Hellwig *et al.*, 1989d).

In a range finding study, groups of 10 female Wistar rats received dermal applications of vinclozolin at doses of 0, 300, 900, or 2500 mg/kg bw per day for 6 h/day on days 6-19 of gestation. The substance was applied onto the dorsal area of the trunk under an occlusive dressing, and the animals were killed on day 20. Adrenal and liver weights were increased, but not in a dose-related fashion, in all treated animals. The anogenital distances were reduced in male fetuses but the effect was not dose-related. The incidences of dilated renal pelvis in fetuses were increased in a dose-related fashion at all doses, with statistical significance at 900 and 2500 mg/kg bw per day. The absence of a dose-response relationship for some of the effects observed in this study is probably attributable to limited dermal absorption of the rather pasty suspension of vinclozolin at 2500 mg/kg bw per day.

In the main study, groups of female Wistar rats received dermal applications of 0, 60, 180, or 360 mg/kg bw per day for 6 h/day on days 6-19 of gestation. The substance was applied on the dorsal area of the trunk under an occlusive dressing, and the animals were killed on day 20. There were no deaths, no abortions, no signs of toxicity,

no treatment-related effects on body weight or food consumption, and no treatment-related findings in haematological investigations performed on day 20. Absolute liver weights were increased at 180 and 360 mg/kg bw per day; absolute adrenal weights were significantly increased at these doses but not dose-dependently. There were no treatment-related macroscopic pathological findings or effects on implantation losses, resorptions, numbers of fetuses, sex ratio, or weights of fetuses or placentae. The anogenital distances of males at

180 and 360 mg/kg bw per day were decreased, but there were no other treatment-related malformations, variations, or retardations in treated fetuses. The NOAEL for maternal toxicity was 60 mg/kg bw per day, on the basis of increased absolute adrenal and liver weights at 180 and 360 mg/kg bw per day. The NOAEL for fetotoxicity was > 360 mg/kg bw per day, and that for teratogenicity was 60 mg/kg bw per day, on the basis of reductions in anogenital distances in male fetuses at 180 and 360 mg/kg bw per day (Hellwig *et al.,* 1990b).

Rabbits

Groups of 15 New Zealand white female rabbits were treated orally with vinclozolin at doses of 0, 20, 80, or 300 mg/kg bw per day on days 6-18 of gestation and were killed on day 29. There were no signs of toxicity and no deaths that were obviously attributable to treatment. Body-weight gain was not affected, and there were no treatment-related effects on numbers of live young, sex ratio, embryonic deaths, pre-implantation losses, or litter or fetal weights. The frequency of post-implantation losses was slightly increased but was within historical control values. There were no treatment-related major malformations or visceral anomalies. There was a slight, non-dose-dependent increase in the incidence of minor skeletal anomalies, which was within historical control values. The NOAEL for maternal toxicity and fetotoxicity was > 300 mg/kg bw per day (Cozens *et al.,* 1981; Cozens & Palmer, 1987).

Groups of 15 female Himalayan rabbits were treated orally with vinclozolin at 0, 50, 200, or 800 mg/kg bw per day on days 7-28 of gestation and were killed on day 29. One female at 200 mg/kg bw per day aborted on day 26 and was killed; at 800 mg/kg bw per day, one female died, one aborted and died, and 11 aborted and were killed. The abortions occurred between days 21 and 27. Clinical signs of toxicity included reddish-brown discolouration of the urine in 13 females and apathy, hunched posture, conjunctivitis, and urine-smeared fur in one or two animals at 800 mg/kg bw per day. The female at 200 mg/kg bw per day which aborted had vaginal haemorrhage and discoloured urine. A second female at this dose had vaginal haemorrhage on day 24. The food consumption of animals at 800 mg/kg bw per day was significantly

reduced during the treatment period, and that of animals at 200 mg/kg bw per day was reduced mainly on days 7-19. Animals at 50 and 200 mg/kg bw per day had no treatment-related increases in pre- or post-implantation losses. The only dam at 800 mg/kg bw per day with viable fetuses had three post-implantation losses, as early resorptions. There were no treatment-related resorptions in animals at 50 or 200 mg/kg bw per day. The sex ratios, numbers of live fetuses, and placental and fetal weights were not affected by treatment at 50 or 200 mg/kg bw per day. There were no treatment-related external malformations or visceral abnormalities. At 800 mg/kg bw per day, one of four viable fetuses had malformations of the sternebrae, but the relevance of this finding is questionable owing to the small number of fetuses. One of 94 fetuses at 50 mg/kg bw per day also had malformations of the sternebrae, but the incidence was within the historical control range of 0-1% (mean, 0.2%). No treatment-related changes in the male fetal genital organs were observed. The NOAEL for maternal toxicity was 50 mg/kg bw per day, on the basis of reduced food consumption, abortion, and clinical signs of toxicity at 200 mg/kg bw per day. The NOAEL for embryo- and fetotoxicity was 200 mg/kg bw per day, on the basis of three resorptions as a consequence of maternal toxicity in the female alive at termination in the high-dose group. There was no evidence of teratogenicity at 50 or 200 mg/kg bw per day (Hellwig *et al.,* 1990c).

Groups of 20 female Himalayan rabbits were treated orally with vinclozolin at 0 or 400 mg/kg bw per day on days 7-8 of gestation and were killed on day 29. One female at 400 mg/kg bw per day aborted and died on day 19, and nine aborted and were killed between days 20 and 27. Clinical signs of toxicity included blood in the bedding of two treated females, one of which aborted. Eight had red-brown urine. The food consumption of treated animals was reduced on days 7-26, and body-weight gain was impaired on days 7-25. Pre- and post-implantation losses were increased, and the number of early resorptions was thus also increased. The numbers of live fetuses per litter were slightly decreased, and the ratio of males:females was 1:1.8 at 400 mg/kg bw per day in comparison with 1:1.16 in the controls; however, no

treatment-related changes were seen in male fetal genital organs. Fetal weights were increased in the treated animals, probably as a consequence of the slightly decreased litter sizes. There were no external or soft-tissue malformations, but the frequency of separated origins of the carotids was 36% in the treated group in comparison with 10% in concurrent controls and a mean of 19% in historical controls (range, 10-31%). There were no skeletal malformations and no treatment-related skeletal variations or retardations; in particular, no malformed sternebrae were observed, indicating that the effect observed in the previous study at 50 mg/kg bw per day was not treatment-related. The NOAEL for maternal, embryo-, and fetotoxicity was < 400 mg/kg bw per day. There was no clear evidence of teratogenicity, but the increased incidence of separated origins of the carotids indicates potential teratogenicity at this severely maternally toxic dose (Hellwig *et al.,* 1990d).

(f) Genotoxicity

The results of standard regulatory assays for genotoxicity are summarized in Table 3.

A medium-term bioassay based on preneoplastic glutathione *S*-transferase placental form (GST-P)-positive foci in rat liver was performed with vinclozolin. Rats were injected with *N*-nitrosodiethylamine and two weeks later were fed a diet containing 2000 ppm vinclozolin for six weeks, with partial hepatectomy at week 3; they were then killed. In one group that received only vinclozolin, negative results were obtained, but administration after initiation with *N*-nitrosodiethylamine gave positive results. The authors reported that the test is highly predictive for genotoxic hepatocarcinogens but less predictive for carcinogens that have target organs other than the liver. They suggested that since the assay is based on the two-stage hypothesis of carcinogenesis, chemicals for which the results are positive are tumour promoters in rat liver (Ito & Hasegawa, 1992; Ito *et al.,* 1993, 1994).

Vinclozolin was cytotoxic in BALB/c3T3 cells in the absence but not in the presence of an exogenous metabolic system. It induced transformation in this cell line in both the presence and the absence of metabolic activation (Perocco *et al.,* 1993).

As shown in Table 3, negative results were obtained in a range of assays *in vivo* and *in vitro.* Although positive results were

obtained in the medium-term bioassay and in the test for cell transformation, these studies are not well validated.

(g) Special studies

(i) Dermal and ocular irritation and dermal sensitization

The intact and abraded dorsal skin of four male and two female white Vienna rabbits was treated with a 50% aqueous suspension of vinclozolin under an occlusive covering for 24 h. The intact skin of five of the six animals had well-defined erythema, and slight oedema was seen in one animal. All of the reactions subsided completely within 72 h. The erythema that formed on the abraded skin of all animals after 24 h was more severe than that on the intact skin; it had completely subsided in one-half the animals by 72 h but remained unchanged in the other half. One animal in this group had slight oedema after 24 h but no longer at 72 h. Vinclozolin cannot be classified as a skin irritant, however, a slight, transient irritation potential was evident (Hildebrand, 1977a).

Table 3. Results of tests for the genotoxicity of vinclozolin

End-point	Test system	Concentration or dose	Purity (%)	Results	Reference
In vitro					
Reverse mutation	S typhimurium TA98, TA100, TA1537	≤ 1000 µg/plate	92.8	Negative[a]	Oesch (1977)
Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538, E. coli WP hrc	≤ 3000 µg/plate	92.8	Negative[a]	Shirasu et al. (1977)
Reverse mutation	S. typhimurium TA98, TA100 TA1535, TA1537, TA1538	≤ 10 000 µg/plate	98.1	Negative[a]	Gelbke & Engelhardt (1983)
Gene mutation hprt	L5178Y cells	≤ 1000 µg/ml	NR	Negative[a]	Witterland & Hoorn (1984)
Gene mutation hprt	Chinese hamster ovary cells	≤ 10 mg/ml	> 99.5	Negative[a]	Gelbke & Jackh (1975)
Chromosomal aberration	Chinese hamster ovary cells	≤ 500 µg/ml	NR	Negative[a]	Murli (1989)
DNA repair	B. subtilis M45 and H17 rec	≤ 2000 µg/plate	92.8	Negative	Shirasu et al. (1977)
DNA repair	B. subtilis M45 and H17 rec	≤ 10 000 µg/plate	NR	Negative[a]	Hoorn (1983)

Unscheduled DNA synthesis	Rat hepatocytes	≤ 1000 µg/ml	> 99.5	Negative	Cifone & Myhr (1984)

In vivo

Host-mediated mutation	S. typhimurium G46 his^-	2 × 200 and 1000 mg/kg bw	92.8	Negative	Shirasu et al. (1977)
Dominant lethal mutation	NMRI male mice	5 × 2000 mg/kg bw	NR	Negative	Hofmann & Peh (1975b)
Sister chromatid exchange	Male and female Chinese hamsters	3830 and 5620 mg/kg bw	98.1	Negative	Gelbke & Engelhardt (1981)

NR, not reported

[a] With and without metabolic activation

Eye irritation was investigated in three male and three female New Zealand white rabbits. After 24 h, the only finding was slight redness of the conjunctivae, which was not completely reversible within 72 h Vinclozolin may be regarded as not irritating to the eyes (Hildebrand, 1977b).

Technical-grade vinclozolin was tested in groups of 12 male Pirbright white guinea-pigs and five controls in the Magnusson-Kligman maximization test. The treated animals had questionable dermal changes after the first challenge and distinct changes after the second, suggesting sensitization (Gelbke, 1979).

No dermal sensitization was observed in an open epicutaneous test in guinea-pigs with BAS 352 04 F formulation (Ronilan), containing 50% vinclozolin. A 60% preparation in water caused slight erythema and oedema at the beginning of the induction period; a 20% preparation elicited sporadic skin irritation. Since no differences were found between the control and test groups, these concentrations are assumed not to be sensitizing. Neither skin irritation nor sensitization was observed with 2 or 6% preparations. These concentrations can be assumed not to have sensitizing potential that would be of importance under field conditions (Grundler & Gelbke, 1980).

(ii) Hormonal effects

Groups of 20 male and 20 female Wistar rats were fed diets containing 4500 ppm vinclozolin for six months; the control group consisted of 10 males and 10 females. After sacrifice, blood was

collected and the levels of the following hormones were determined:
adrenocorticotrophic hormone (ACTH), corticosterone, dehydroepian-
dosterone (DHEA), testosterone, estradiol, hydroxy-progesterone, and
luteinizing hormone (LH). Males showed more effects than females: in
males, the level of LH was increased by about 10-fold and those of
testosterone and DHEA doubled; in females, the level of LH was
increased by 2.5-fold, but there was no change in the concentrations
of sex steroids. That of ACTH increased in animals of each sex, but
the results were variable and the relationship of this change to
treatment is uncertain (Knuppen, 1989).

Groups of 20 male and 20 female Wistar rats were treated with 0
or 4500 ppm vinclozolin for three months, and groups of 10 of each sex
were then allowed to recover for three months to study the
reversibility of any changes. The hormones that were measured were LH,
follicle-stimulating hormone, testosterone, estradiol, DHEA, ACTH,
aldosterone, and corticosterone. The results basically confirmed those
of the previous study. The levels of LH, follicle-stimulating hormone,

testosterone, and DHEA were increased in males, LH most markedly,
while only that of LH was increased in females. The concentration of
ACTH was increased in both males and females, and those of aldosterone
and corticosterone were marginally increased in males but decreased in
females. All of these changes, except perhaps that of ACTH in females,
was reversible within two months (Mellert *et al.*, 1992).

(iii) Receptor binding

In a study of binding to the androgen receptor, vinclozolin was
incubated *in vitro* with MCF-7 cells derived from a human mammary
carcinoma which contains a large amount of androgen receptor. A clear
affinity with cytosolic and nuclear androgen receptors was seen
(Knuppen, 1990). In order to confirm this result and to investigate
whether the binding is due to vinclozolin or its main rat metabolite
(Reg. No. 119 208), their effects were compared with those of the
anti-androgenic drug Flutamide and the synthetic androgen Mibolerone.
Vinclozolin bound to the androgen receptor with an affinity of
approximately 50% of that of Flutamide, and the binding affinity of
the metabolite was virtually zero (Knuppen & Schutze, 1991). In a

comparison of the effects of vinclozolin on receptors *in vitro* and *in vivo,* not reported in detail, vinclozolin was shown to have a binding affinity similar to that of Flutamide for androgen receptors in the LNCAP cell line, which is derived from human prostate, a typical target tissue for vinclozolin-mediated anti-androgenic effects. Vinclozolin was also capable of binding to androgen receptors in castrated Wistar rats (Knuppen & Schutze, 1992).

The ability of vinclozolin to inhibit 5-alpha-reductase and to compete with androgen for binding at the androgen receptor was investigated. Neither vinclozolin nor its degradation products 2-{[(3,5-dichlorophenyl)carbamoyl]oxy}-2-methyl-3-butenoic acid and 3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide inhibited 5-alpha-reductase. Although vinclozolin competed only weakly with androgen for binding at the androgen receptor, the two metabolites were effective antagonists. The authors reported that the concentrations of the first metabolite in the serum of pregnant rats after treatment with 100 mg/kg bw per day vinclozolin could be sufficient to meet or exceed the K_i for androgen receptor inhibition *in vitro* (Kelce *et al.,* 1994).

(iv) Nephrotoxicity

The acute nephrotoxic potential of vinclozolin was compared with that of two other *N*-(3,5-dichlorophenyl) carboximide fungicides: *N*-(3,5-dichlorophenyl) succimide and iprodione. Groups of four male Fischer 344 rats received a single intraperitoneal injection of 0.4 or 1.0 mmol/kg bw or vehicle, and renal function was monitored at 24 and 48 h. *N*-(3,5-Dichlorophenyl) succimide induced renal effects characterized by marked diuresis, increased proteinuria, elevated blood urea nitrogen, increased kidney weights, and proximal tubule necrosis. Iprodione and vinclozolin caused only minor or no alterations in renal function (Rankin *et al.,* 1989).

(v) Haemoglobin adduct formation

Female Wistar rats were treated orally with various pesticides at doses of up to 1 mmol/kg bw. Blood was taken 24 h after treatment, haemoglobin was isolated and hydrolysed with 1N sodium hydroxide, and aromatic amines extracted and quantified. No adducts with 3,5-di-chloroaniline from vinclozolin were found (Sabbioni & Neumann, 1990).

(vi) Review of ophthalmoscopic findings

In a review of the cataracts and other lenticular changes seen at ophthalmoscopy in short- and long-term studies with vinclozolin in rats, an analysis was carried out to determine whether these changes were due to acceleration of normal, age-related changes, since lenticular changes are seen commonly in old rats and cataracts also occur spontaneously in untreated rats. The clear NOAELs are shown in Table 4. Vinclozolin induced cataracts and other lenticular changes only in rats, and there were no treatment-related lenticular changes in B6C3F1 or C57B1 mice or in dogs. In the studies of absorption and distribution, a high concentration of radiolabel was found in the Harderian gland, which is situated close to the eye and secretes a fluid onto its surface. The presence of radiolabel in this secretion was confirmed by examination of enlargements of whole-body autoradiographs (Schilling, 1993).

3. Observations in humans

A cross-sectional study was performed of 67 men who had handled vinclozolin for 1-13 years during its synthesis and formulation and 52 unexposed controls. The men were monitored by determining urinary metabolites containing a 3,5-dichloraniline moiety and observation for reversible changes in the levels of hormones of the adrenocortico-tropic and gonadotropic feedback systems, signs of liver injury, haemolytic anaemia, cataract formation, and hormonally induced hyperplasia and tumours at high doses. The clinical investigation consisted of a medical and occupational history questionnaire, physical examination, laboratory determinations, including measurements of testosterone, LH, and follicle-stimulating hormone, ultrasonography of the liver and prostate, a detailed examination, and routine spirometry. The mean 3,5-dichloraniline concentration in exposed workers was 235-422 µg/g creatinine, depending on the work area, in comparison with a mean of 7 µg/g creatinine in controls. On the basis of a series of assumptions, including 40% excretion through the kidneys, 1.5-litre urine output per day, and 70-kg body weight,

the authors estimated that the occupational exposure of two-thirds of the employees to vinclozolin exceeded 25 µg/kg bw per day. The

physical examinations and laboratory tests provided no evidence of vinclozolin-induced hormonal responses, liver injury, prostatic changes, cataract formation, or haemolytic anaemia. The authors concluded that vinclozolin induced no health effects and, in particular, no anti-androgenic effects (Zober *et al.,* 1995).

Table 4. NOAELs for ophthalmoscopic effects in short- and long-term studies of the toxicity of vinclozolin in rats

Effect	NOAEL (ppm)	
	Short-term study	Long-term study
Cataracts	1000	150
Striations	300	150
Bosselated lens structure	1000	150
Bulbiform thickening	3000	150
Opacities	3000	150

Comments

Vinclozolin is well absorbed after oral administration to rats and extensively metabolized. The majority of the administered radiolabel was found in the bile, and no unchanged vinclozolin was excreted in the urine. After single oral doses of radiolabelled vinclozolin, excretion was rapid; after multiple doses there was no significant accumulation. Vinclozolin is only moderately absorbed via the dermal route in rats: over 72 h, about 17% of a dose of 0.13 mg/kg bw was excreted in the urine.

Vinclozolin has low acute toxicity, with an oral LD_{50} in rats of > 15 000 mg/kg bw. The clinical signs of toxicity after acute dosing with vinclozolin were generally non-specific and there were no consistent treatment-related findings at necropsy. Vinclozolin is not irritating to rabbit skin or eyes, but induced skin sensitization in a maximization study in guinea-pigs. WHO has classified vinclozolin as unlikely to present an acute hazard in normal use.

Studies of repeated administration were carried out in mice, rats, rabbits and dogs, in which vinclozolin and/or its metabolites caused toxic effects indicative of anti-androgenic activity. In two

three-month feeding studies in different strains of mice at dietary levels of 100-5000 ppm, the NOAEL was equivalent to 20 mg/kg bw per day, on the basis of signs of hepatotoxicity, signs consistent with anti-androgenicity, and changes in the adrenal glands. In two recent three-month feeding studies in rats (at levels of 0, 300, 1000, and 3000 ppm and 0 and 50 ppm, respectively) vinclozolin caused changes qualitatively similar to those seen in mice; however, effects on the adrenal glands (including lipidosis) were seen at 300 ppm, and the NOAEL was confirmed in the second study as 50 ppm, equal to 4 mg/kg bw per day. In a 12-month feeding study in dogs at dietary levels of 0, 35, 75 150, or 1500 ppm, the NOAEL was 75 ppm, equal to 2.4 mg/kg bw per day, on the basis of pathological changes in the liver, spleen, prostate, testis, and adrenals. The results of studies incorporating withdrawal periods indicate that the anti-androgenic effects of vinclozolin are reversible on cessation of treatment.

In a recent study of carcinogenicity in C57Bl/6 mice at dietary levels of 0, 15, 150, 3000, or 8000 ppm, hepatocellular carcinomas were seen at 8000 ppm. There was evidence of toxicity at 3000 ppm, including hepatotoxicity, Leydig-cell hyperplasia, atrophy of accessory sex glands, atrophic uteri, and lipidosis in the cortico-medullary region of the adrenals. The NOAEL was 150 ppm, equal to 24 mg/kg bw per day. In an earlier study in NMRI mice at levels of 0, 160, 490, 1460, or 4370 ppm, survival was adversely affected at the highest dose, and the NOAEL was 490 ppm on the basis of increased liver weight, without histological change. In rats, the long-term toxicity and carcinogenicity of vinclozolin has recently been investigated in three studies with dietary levels of 25-4500 ppm.

Cataracts and other lenticular changes were seen in rats treated with 50 ppm or more. (Mice and dogs were closely examined for ocular changes, but vinclozolin did not affect the eyes in these species.) An increased incidence of Leydig-cell tumours was seen in rats treated with 150 ppm and more, together with atrophy of accessory sex glands. Benign sex cord stromal tumours in the ovaries were seen in rats treated at 500 ppm and above, and uterine adenocarcinomas were detected at 3000 ppm (the highest dose tested in the carcinogenicity study). Adrenal tumours were seen at 1500 ppm and above.

Hepatocellular carcinomas were seen in males treated with 4500 ppm, and signs of hepatotoxicity were seen in rats treated with 150 ppm or more. The NOAEL was 25 ppm, equal to 1.4 mg/kg bw per day.

In multi-generation studies, vinclozolin led to infertility of males, owing to feminization of the outer genital organs, at dietary levels of 1000 ppm or more. At 300 ppm, although all males were eventually proved fertile, the observed effects may have indicated sub-fertility. At 50 ppm, the only adverse effect was a reduction in epididymal weight (with no associated morphological changes) in F_2 offspring. The NOAEL was 40 ppm, equivalent to approximately 4 mg/kg bw per day. Recent investigations of developmental toxicity have been conducted in rats and rabbits. In rats, the most sensitive indicator of teratogenicity was a reduction in the anogenital distance; in a series of studies, the NOAEL for a change in anogenital distance was 15 mg/kg bw per day. The NOAEL for fetotoxicity was about 100 mg/kg bw per day, on the basis of signs of developmental delay, while the NOAEL for maternal toxicity was about 400 mg/kg bw per day, on the basis of clinical signs of toxicity.

Three studies of developmental toxicity have been conducted in rabbits. In the first, there were no signs of maternal toxicity, fetotoxicity, or teratogenicity at doses up to and including 300 mg/kg bw per day. In the second study, with doses up to and including 800 mg/kg bw per day, toxicity led to extensive mortality at the highest dose, precluding any reliable assessment at this dose. The NOAEL for maternal toxicity was 50 mg/kg bw per day, and that for fetotoxicity was 200 mg/kg bw per day; there was no evidence of teratogenicity at this dose (the highest dose available for assessment). The third study involved only one dose, 400 mg/kg bw per day. The number of female offspring exceeded the number of males, but there was no treatment-related change in the appearance of the male fetal genital organs. An increase in the incidence of separated origins of the carotid arteries indicated potential teratogenicity at this maternally toxic dose.

Vinclozolin has been tested for genotoxicity in a range of tests *in vivo* and *in vitro.* The Meeting concluded that vinclozolin is

not genotoxic. It noted that positive results were obtained in a study of cell transformation, but the process giving rise to this effect is unknown. One study suggests that vinclozolin may be a promoter in rat liver *in vivo,* which may indicate the mechanism by which liver tumours were induced in rats at a high dose.

Studies have been conducted that confirm the anti-androgenic properties of vinclozolin, which are likely to be associated with binding to the androgen receptor. This proposed mechanism of action could account for the results seen in studies of the reproductive toxicity and long-term toxicity of vinclozolin.

In an epidemiological study of manufacturing plant personnel, it was concluded that there was no evidence that vinclozolin had induced health effects in employees with possible long-term exposure.

An ADI of 0-0.01 mg/kg bw was established on the basis of the NOAEL of 1.4 mg/kg bw per day in the two-year study of carcinogenicity in rats and a safety factor of 100.

Toxicological evaluation

Levels that cause no toxic effect

Mouse:	100 ppm, equal to 20 mg/kg bw per day (three-month study of toxicity)
	490 ppm, equivalent to 63 mg/kg bw per day (112-week study of toxicity and carcinogenicity in NMRI mice)
	150 ppm equal to 24 mg/kg bw per day (18-month study of toxicity and carcinogenicity in C57Bl/6 mice)
Rat:	50 ppm, equal to 4 mg/kg bw per day (three-month study of toxicity)
	25 ppm, equal to 1.4 mg/kg bw per day (two-year study of toxicity and carcinogenicity)
	40 ppm, equivalent to 4 mg/kg bw per day (study of reproductive toxicity)
	15 mg/kg bw per day (study of developmental toxicity)
	100 mg/kg bw per day (fetotoxicity in a study of developmental toxicity)
	400 mg/kg bw per day (maternal toxicity in study of developmental toxicity)
Rabbit:	50 mg/kg bw per day (maternal toxicity in study of developmental toxicity)
	200 mg/kg bw per day (fetotoxicity in a study of developmental toxicity)

Dog: 75 ppm, equal to 2.4 mg/kg bw per day (one-year study of toxicity)

Estimate of acceptable daily intake for humans

0-0.01 mg/kg bw

Studies that would provide information useful for continued evaluation of the compound

Further observations in humans

Toxicological criteria for setting guidance values for dietary and non-dietary exposure to vinclozolin

Exposure	Relevant route, study type, species	Results, remarks
Short-term (1-7 days)	Oral, toxicity, rat	LD_{50} > 15 000 mg/kg bw
	Dermal, toxicity, rat	LD_{50} > 5000 mg/kg bw
	Dermal, irritation, rabbit	Not irritating
	Ocular, irritation, rabbit	Not irritating
	Dermal, sensitization, guinea-pig	Sensitizing in maximization test
	Inhalation, toxicity, rat	LC_{50} > 29 mg/litre air
Mid-term (1-26 weeks)	Oral, developmental toxicity, rat	NOAEL = 15 mg/kg bw per day; teratogenicity
Long-term (≥ one year)	Dietary, two years, toxicity and carcinogenicity, rat	NOAEL = 1.4 mg/kg bw per day; signs of antiandrogenicity
	Dietary, one year, toxicity, dog	NOAEL = 2.4 mg/kg bw per day; signs of antiandrogenicity

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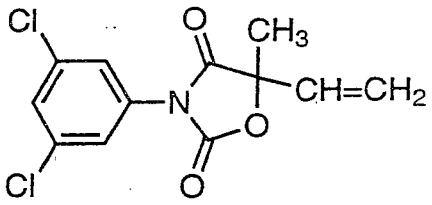
techniques. Efficacy assays are made against *Aphis fabae* for 'Vertalec', and scales of *Trialeurodes vaporariorum* for 'Mycotal'.

MAMMALIAN TOXICOLOGY
No skin or eye irritation observed. There is no evidence of acute or chronic toxicity, infectivity or hypersensitivity to mammals. No allergic responses or health problems have been observed by research workers, manufacturing staff or users.

vinclozolin

Fungicide

dicarboximide



NOMENCLATURE
Common name vinclozolin (BSI, E-ISO, JMAF), vinclozoline ((*m*) F-ISO).
IUPAC name (*RS*)-3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione.
C.A. name (±)-3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione.
CAS RN *[50471-44-8]* unstated stereochemistry **Development code** BAS 352F.

PHYSICO-CHEMICAL PROPERTIES
Composition Tech. is ≥93% *m/m* pure.
Mol. wt. 286.1 **Mol. formula** $C_{12}H_9Cl_2NO_3$
Form Colourless crystals with a slight aromatic odour. **M.p.** 108 °C (tech.)
B.p. 131 °C/0.05 mm Hg **V.p.** 0.016 mPa (20 °C) **SG/density** 1.51 **K_{ow}** 1000 (pH 7) **Solubility** In water at 20 °C, 3.4 mg/l. In ethanol 14, acetone 435, ethyl acetate 253, cyclohexane 9, diethyl ether 63, benzene 146, xylene 110, cyclohexanone *c.* 540, chloroform 319 (all in g/kg at 20 °C). **Stability** Stable up to 50 °C. Stable in neutral and weakly acidic media. In 0.1N sodium hydroxide, 50% hydrolysis occurs in 3.8 hours.

COMMERCIALISATION
History Fungicide reported by E. - H. Pommer & D. Mangold (*Meded. Fac. Landbouwwet. Rijksuniv. Gent*, 1975, **40**, 713). Introduced in Germany (1976) by BASF AG. **Patents** DE 2207576 **Manufacturer** BASF.

APPLICATIONS
Mode of action Non-systemic fungicide with protective action. Prevents spore germination.
Uses Control of *Botrytis* and *Sclerotinia* spp. in vines, strawberries, oilseed rape, vegetables, fruit, and ornamentals; *Monilia* spp. in pome fruit and stone fruit; *Sclerotinia*, *Helminthosporium*, and *Corticium* spp. in turf, etc. **Phytotoxicity** Non-phytotoxic.
Formulation type WP; SC; DP; FD. **Compatibility** Compatible with many other pesticides.

Principal tradename 'Ronilan' (BASF). **Mixtures** [*vinclozolin* +] carbendazim; chlorothalonil; maneb; sulfur; thiophanate-methyl; thiram.

ANALYSIS

Product analysis by glc with FID (*CIPAC Handbook,* 1988, **D,** 173). **Residues** determined by hydrolysis to 3,5-dichloroaniline, a derivative of which is measured by glc with ECD (*Methodensammlung Rückstandsanal. Pflanzenschutzmitteln,* 1987, **XII,** 6, S8, S19; *Anal. Methods Residues Pestic.,* 1988, Part I, M1, M12; A. Ambrus *et al., J. Assoc. Off. Anal. Chem.,* 1981, **64,** 733). Details available from BASF.

MAMMALIAN TOXICOLOGY

Reviews *Pesticide residues in food - 1988.* FAO Plant Production and Protection Paper 92, 1988. *Pesticide residues in food - 1988 evaluations. Part II - Toxicology. FAO Plant Production and Protection Paper 93/2, 1989.* **Acute oral** LD_{50} for rats and mice > 10000, guinea pigs *c.* 8000 mg/kg. **Skin and eye** Acute percutaneous LD_{50} for rats > 2500 mg/kg. Moderate skin irritant; slight mucous membrane irritant (rabbits). **Inhalation** LC_{50} (4 h) for rats > 29.1 mg/l air. **NOEL** for male rats 27.1 mg/kg b.w., for female rats 28.2 mg/kg b.w.; (90 d) for rats 450 mg/kg diet, for dogs 300 mg/kg diet. **ADI** (JMPR) 0.07 mg/kg b.w. [1988]. **Toxicity class** WHO Table 5; EPA IV.

ECOTOXICOLOGY

Birds Acute oral LD_{50} for quail > 2510 mg/kg. LC_{50} for quail > 5620 mg/kg. **Fish** LC_{50} (96 h) for trout 22-32, guppies 32.5, bluegill 50 mg a.i. (as WP)/l. **Bees** Not toxic to bees. **Daphnia** EC_{50} (48 h) 4.0 mg/l. **Other beneficial spp.** Not toxic to earthworms.

ENVIRONMENTAL FATE

Animals In the hen, the major metabolic routes are epoxidation of the vinyl group, followed by hydration of the intermediate epoxide, and by hydrolytic cleavage of the heterocyclic ring (G. M. Dean *et al., Proc. Br. Crop Prot. Conf. - Pests Dis.* 1988, **2,** 693-8). In rats, following oral administration, eliminated in approximately equal proportions in the urine and faeces, with the principal metabolite being *N*-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutanamide. **Plants** In plants, the primary metabolites are (1-carboxy-1-methyl)allyl 3,5-dichlorophenylcarbamate and *N*-(3,5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenamide. Alkaline hydrolysis leads to loss of 3,5-dichloroaniline from vinclozolin and its metabolites. The metabolites exist as conjugates.
Soil and water Metabolism occurs by loss of the vinyl group, cleavage of the 5-membered ring and eventual formation of 3,5-dichloroaniline. Vinclozolin is rather persistent in soil, being only partly degraded by soil microorganisms. K_{oc} 100-735.