Australian Government

Australian Pesticides and Veterinary Medicines Authority

DIURON

HUMAN HEALTH ASSESSMENT

July 2011

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1. INTRODUCTION

Diuron is a substituted chloranilide derivative with structural similarities to linuron and monuron. Diuron, for use as a herbicide, was first reported by Bucha & Todd (Science, 1951), and introduced under the trademak “Karmex” in 1954 to the US market by E.I. Du Pont de Nemours & Co.

Diuron is a broad-spectrum residual herbicide acting through the inhibition of plant photosynthesis. It is primarily absorbed through plant roots and has a soil half-life of the order of one hundred days.

Diuron is one of some 80 agricultural and veterinary chemicals identified as candidates for priority review under Australia's Chemicals Review Program. The main concerns that led to the nomination of diuron for review related to the potential for environmental contamination, particularly the marine environment. Concerns have also been raised about the possible toxicity of the impurities in the active constituent. A number of data submissions on the toxicology of diuron have been received from industry and these have been assessed in detail.

1.1 History of public health considerations of diuron in Australia

Australian public health standards for agricultural and veterinary chemicals that may enter the food chain include the Poisons Schedule, First Aid and Safety Directions (FAISDs), the human acceptable daily intake (ADI) and the acute reference dose (ARfD). A further regulatory standard called the maximum residue level (MRL) is a measure of the residues present in unprocessed food (eg. grain, meat etc.) and hence is an indicator of good agricultural practice.

From the mid 1950s until 1992, Australian public health standards were set by committee process under the auspices of the NHMRC. “Pesticide Tolerances” in food were first set in 1956 by the Food Additives Committee. Between 1962 and 1966, the Food Additives Committee maintained a Sub-Committee on Pesticides and Agricultural Chemical Residues In Or On Foods (later re-named the Pesticide Residues in Food Sub-Committee), which adopted the then Canadian scheme as a basis for establishing tolerances. From 1967 onwards, Australian MRLs and ADIs for pesticides were established by the Pesticide and Agricultural Chemicals Committee (PACC), until the Department of Health and Ageing became directly responsible for setting ADIs in November 1992. Responsibility for pesticide and veterinary chemical MRLs in food was transferred to the NRA in June 1994, after which the PACC was removed from the control of the NHMRC and re-constituted as the Advisory Committee on Pesticides and Health (ACPH). The ACPH provides the Department of Health and Ageing, the TGA and the APVMA with advice on issues of policy and practice having possible implications for public health and the proper use of chemicals in agriculture and elsewhere.

Poisons Schedules for agricultural and veterinary chemicals, drugs and some other hazardous substances are set by the National Drugs and Poisons Schedule Committee (NDPSC). Originally known as the Committee on Poisons Scheduling, the NDPSC was established in 1955 as a sub-committee of the NHMRC Public Health Committee. The NDPSC publishes its decisions in the Standard for the Uniform Scheduling of Drugs and Poisons, which recommends controls on availability, labelling, packaging and advertising. These are incorporated into and enforced by the various Australian State and Territory legislative systems. In 1994, the NDPSC was transferred from the NHMRC to the Australian Health Ministers’ Advisory Council, and was re-constituted again in 1999 as a Statutory Committee of the Therapeutic Goods Administration.

A third committee formerly involved in chemicals management was the NHMRC Standing Committee on Toxicity (SCOT), which was active between 1985 and 1994. SCOT was responsible for providing specialised advice on complex toxicological matters to all the NHMRC Public Health Committee subordinate committees, including the PACC and NDPSC. In response to referrals from these committees, SCOT undertook evaluation of some drugs, pesticides, food additives, poisons, consumer products, chemicals and other hazardous substances relevant to public health.

The regulatory history of public health considerations of diuron by Australian regulatory committees is summarised below.

Date	Regulatory Activity
February 1980	PACSC: MRL recommended for water (0.04 mg/L).
May 1986	PACSC: Recommended maximum levels for TCAB and TCAOB.
November 1987	NDPSC: Considered review of submission with significant toxicological data from Du Pont and Koor Intertrade, and confirmed the scheduling status (exemption, Appendix B) of diuron.
February 1988	NDPSC: Considered the NHMRC's recommendation and agreed the limits of impurity levels of TCAB (20 mg/kg) and TCAOB (2 mg/kg) be included in Appendix L.
August 1990	NDPSC: Considered each of the applications for diuron TGAC clearance based on the levels of microcontaminants TCAB and TCAOB. Suspended consideration of Bayer's application due to bladder hyperplasia and carcinoma in 26-week and 2-year rat studies, and equivocal results from an *in vitro* UDS test in rat bladder epithelium, and a potential association with TCAB and TCAOB.
August 1990 – August 1992	PACSC: Repeatedly considered and finally accepted the applicant's statements on levels of impurities TCAB and TCAOB, and agreed to TGAC clearance.

PACCS – Pesticides and Agricultural Chemical Standing Committee; NDPSC - National Drugs and Poisons Scheduling Committee.

ADI

The current ADI for diuron is 0.006 mg/kg bw/d. This ADI was established in 1987 and derived from a NOEL of 0.625 mg/kg bw/day for abnormal blood pigment seen in a 2-year dog study (Hodge & Downs, 1964), and using a 100 fold safety factor.

Poisons Scheduling

Diuron is currently in Appendix B (Substances considered not to required control by scheduling) of the SUSDP (Confirmed by the NDPSC in November 1987).

Drinking Water Quality Guidelines

The Australian Drinking Water Guidelines (ADWG, 1996) are a joint publication of the National NH&MRC and Agricultural and Resource Management Council of Australia and New Zealand (see *http://www.nhmrc.gov.au/publications/synopses/eh19syn.htm*). The ADGW are not legally enforceable but rather provide a standard for water authorities and State health authorities to ensure the quality and safety of Australia's drinking water.

The Guideline Value (mg/L) is analogous to an MRL in food and is generally based on the analytical limit of determination. It is set at a level consistent with good water management practice and that would not result in any significant risk to the consumer over a lifetime of consumption. If a pesticide is detected at or above this value then the source should be identified and action taken to prevent further contamination. A guideline value is not established for diuron, since it is one of the pesticides that have either been detected on occasions in Australian drinking water or their likely use would indicate that they may occasionally be detected.

The Health Value (also expressed as mg/L) is intended for use by health authorities in managing the health risks associated with inadvertent exposure such as a spill or misuse of a pesticide. The health values are derived so as to limit intake from water alone to approximately 10% of the ADI, on the assumption that (based on current knowledge) there will be no significant risk to health for an adult weighing 70 kg having a daily water consumption of 2 L over a lifetime. The current Health Value for diuron is 0.03 mg/L.

1.2 International Toxicology Assessments

Diuron products are registered in many countries, including New Zealand, United States, Canada, Europe and the United Kingdom.

In 1996, the US EPA has scheduled a re-registration of diuron to be conducted in 2002 under the Federal Insecticides, Fungicides and Rodenticides Act, which requires review of chemicals registered before November 1984. By the end of 2004, the US EPA was scheduled to complete its review of public comments on the preliminary risk assessments and issued its risk management decision and re-registration eligibility decision document for comment (Federal Register: April 21, 2004, Volume 69, Number 77). The current US Oral Reference Dose (RfD) is 0.003 mg/kg bw/day.

Diuron was to be reviewed in the second stage of the European Union (EU) review program that was considering all chemicals registered in the EU before 26 July 1993. The data submission for the second stage of the EU review closed on 30 April 2002. The review is to be conducted by the Danish agricultural chemical regulator. A final report is not yet available. Diuron is currently approved in the UK, but it has been banned in Sweden since 1993 for health and environment reasons.

In recent years, Denmark has banned the use of diuron in anti-fouling paints for boats in order to protect the aquatic environment. The Statutory Order issued by the Ministry of Environment and Energy stated that it is not permitted to import, sell or use anti-fouling bottom paint containing the biocide diuron on ships shorter than 25 metres (Fact Sheet No 24: Anti-fouling bottom paint, 07/01/2003).

Following an incident where it was claimed that irrigation water contaminated by pesticides from railway tracks had caused crop damage, the German Railways (DB) stopped using diuron for weed control on railway lines in 1996. DB switched to glyphosate and trialled alternate means of control such as superheated steam. The German federal parliament subsequently imposed a legal ban on the use of diuron on railways, due to high levels of diuron in groundwater.

As part of a review of all chemical used in marine anti-foulants, the UK Advisory Committee on Pesticides took the decision to cancel the use of diuron in antifouling treatments at its September 2000 meeting, due to environmental and human health concerns. Concerns regarding human health were raised because the available exposure data showed an insufficient margin of safety when compared with the NOAEL.

There are no ADI or ARfD to be set by the Joint FAO/WHO Meetings on Pesticide Residues (JMPR).

1.3 Chemistry – Technical Active

Approved common name: Diuron

Alternative names: Karmex, Krovar

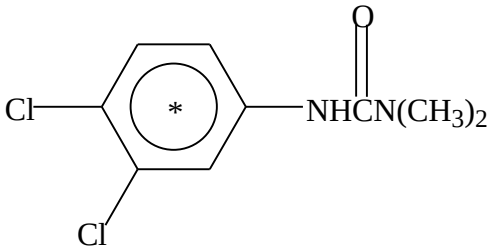
Chemical name: 3-(3,4-dichlorophenyl)-1,1-dimethylurea (IUPAC)
N'-(3,4-dichlorophenyl)-N,N-dimethylurea (CAS)

CAS Registry number: 330-54-1

Empirical formula: $C_9H_{10}Cl_2N_2O$

Molecular weight: 233.1

Chemical structure:



Isotope label: The position of the radiolabel (^{14}C) is indicated by an asterisk*

Chemical class: Phenylurea herbicides

Structural analogues: linuron, monuron, propanil

Chemical and physical properties

Colour:	White
Odour:	None
Physical state:	Crystal
Melting point:	158^{o}C
Partition coefficient: (log K_{ow})	2.68
Vapour pressure:	2 x 10^{-7} mm Hg at 30^{o}C
Water solubility:	42 ppm @ 25 ^{o}C
Stability:	Stable for 2 yrs. In double polyethylene bag inside a fiber drum under warehouse conditions. Metals and metal ion data not given.

Technical active - Declaration of Composition and Batch Analysis

Declarations of composition for technical grade diuron are shown in Appendix III.

Impurities of Toxicological Concern

The minimum compositional standard for active constituent diuron is given in the table below:

Chemical	**Standard**
Diuron	Minimum 950 g/kg
3,3'4,4'-tetrachloroazobenzene	Maximum 20 mg/kg
3,3',4,4'-tetrachloroazoxybenzene	Maximum 2 mg/kg

The maximum impurity levels for diuron were set by Pesticides and Agricultural Chemical Committee in 1986. The Therapeutic Goods Administration (TGA) has indicated concerns regarding two impurities of diuron: 3,3'4,4'-tetrachloroazobenzene (TCAB), 3,3',4,4'-tetrachloroazoxybenzene (TCAOB). The concerns relate to potential carcinogenicity.

The Food and Agriculture Organisation (FAO) of the United Nations does not specify maximum levels for TCAB and TCAOB in the active constituent diuron.

2. METABOLISM AND TOXICOKINETICS

2.1 Rats

Wu D (1996). Absorption, distribution, metabolism and elimination of [^{14}C]-diuron in rats. Lab: XenoBiotic Laboratories, Inc. Plainsboro NJ 08536 & EI du Pont de Nemours and Company Experimental Station, Wilmington DE 19880-0402. Sponsor: & EI du Pont de Nemours and Company, DuPont Agricultural Products, Experimental Station, Wilmington DE 19880-0402. Guidelines: FIFRA. DuPont Report No: AMR 3145-94. Guidelines: US EPA 85-1.

Methods

Sprague-Dawley rats (5/sex/group) received a single oral low dose of 10 mg/kg bw of [^{14}C]-diuron, multiple doses of 10 mg/kg bw/day unlabelled diuron for 14 days followed by 10 mg/kg bw [^{14}C]-diuron on day 15, or a single oral high dose of 400 mg/kg bw [^{14}C]-diuron. A control group (2/sex) received the solvent PEG 400. The rats (Crl: CD BR) were 6-10 weeks old and from Charles River Laboratories in Kingston, NY. Diuron was uniformly labelled in the phenyl ring with a specific activity: 50.2 □Ci/mg. Excreta (urine, cage rinse and faeces) were collected at the time intervals of 0-8, 8-24, 24-48, 48-72 and 72-96 h post dosing. Selected tissues and organs (blood, brain, bone, liver, kidneys, heart, lungs, spleen, fat, muscle, skin, ovaries and uterus or testes, and carcass) were collected from all dose groups at sacrifice at 96 h post dosing. In a preliminary study, the amount of radioactivity recovered in expired air ($^{14}CO_2$) was shown to be minimal, and hence volatiles were not collected in the main study. Levels of radioactivity in the samples were determine by liquid scintillation directly (for urine and cage rinses), or after homogenisation/combustion. Metabolite profiles in pooled samples of urine or faeces were determined by combination of high-performance liquid chromatography, thin-layer chromatography, liquid chromatography/mass spectrometry and liquid scintillation counting.

Results

Diuron was readily absorbed following oral administration. Most of the test substance was excreted as metabolites within the first 24 h post dosing. A total of 94-100% of the dose was excreted in 96 hours. The majority of the administered dose was eliminated in the urine (80-91%), and to a lesser extent in the faeces (8-15%). Metabolites in urine and faeces did not change much with either dosing regime or between the sexes.

Comparison of ^{14}C recovery from rat urine and faeces (%)

Time (h)	10 mg/kg bw (single)				10 mg/kg bw (multiple)				400 mg/kg bw (single)			
	male		female		male		female		male		female	
	urine	faeces	urine	faeces	urine	faeces	urine	faeces	urine	faeces	urine	faeces
0-24	84.6	8.2	84.6	7.0	87.0	8.2	87.0	5.6	47.5	6.7	37.5	2.5
0-48	86.3	9.5	86.5	8.7	88.6	9.3	89.1	7.2	78.3	14.4	78.5	11.3
0-96	87.9	9.9	88.0	9.2	89.9	9.6	91.4	7.7	79.9	15.2	81.6	12.5
Total	97.8		97.2		99.5		99.1		95.1		94.1	

Urine includes cage rinse.

At sacrifice 96 h post dosing, the highest residue levels were generally found in blood, liver and kidneys. Tissues from the high dose group showed proportionally higher residue levels compared to the low dose groups. No accumulation of radioactivity was observed in tissues following repeat dosing.

Comparison of ^{14}C residue in rat tissues and carcasses (ppm)

Tissue	10 mg/kg bw (single)		10 mg/kg bw (multiple)		400 mg/kg bw (single)	
	M	F	M	F	M	F
Blood	0.16	0.29	0.13	0.26	8.93	8.24
Kidneys	0.16	0.30	0.14	0.28	7.12	8.94
Liver	0.13	0.18	0.13	0.16	5.57	6.80
Other tissues	≤ 0.055	≤ 0.068	≤ 0.055	≤ 0.101	≤ 3.369	≤ 5.039

Metabolism of diuron was extensive. Eight metabolites were identified in the urine, and four were in the faeces. The major metabolite found in the rat was IN-R915, and to a lesser extent glucuronide conjugates of IN-U1232, HO-Me-In-D0432, IN-D0230, IN-T1035 and sulphate conjugate of IN-U1232 (only found in low dose groups), as well as free metabolites IN-U1232, IN-JT680, IN-T1035 and IN-KH289 (see the following Table for the codes). In addition, several minor polar and nonpolar metabolites were detected, and only a small amount of diuron (0.1-1.6%) was found in the rat faeces, indicating extensive degradation/biotransformation.

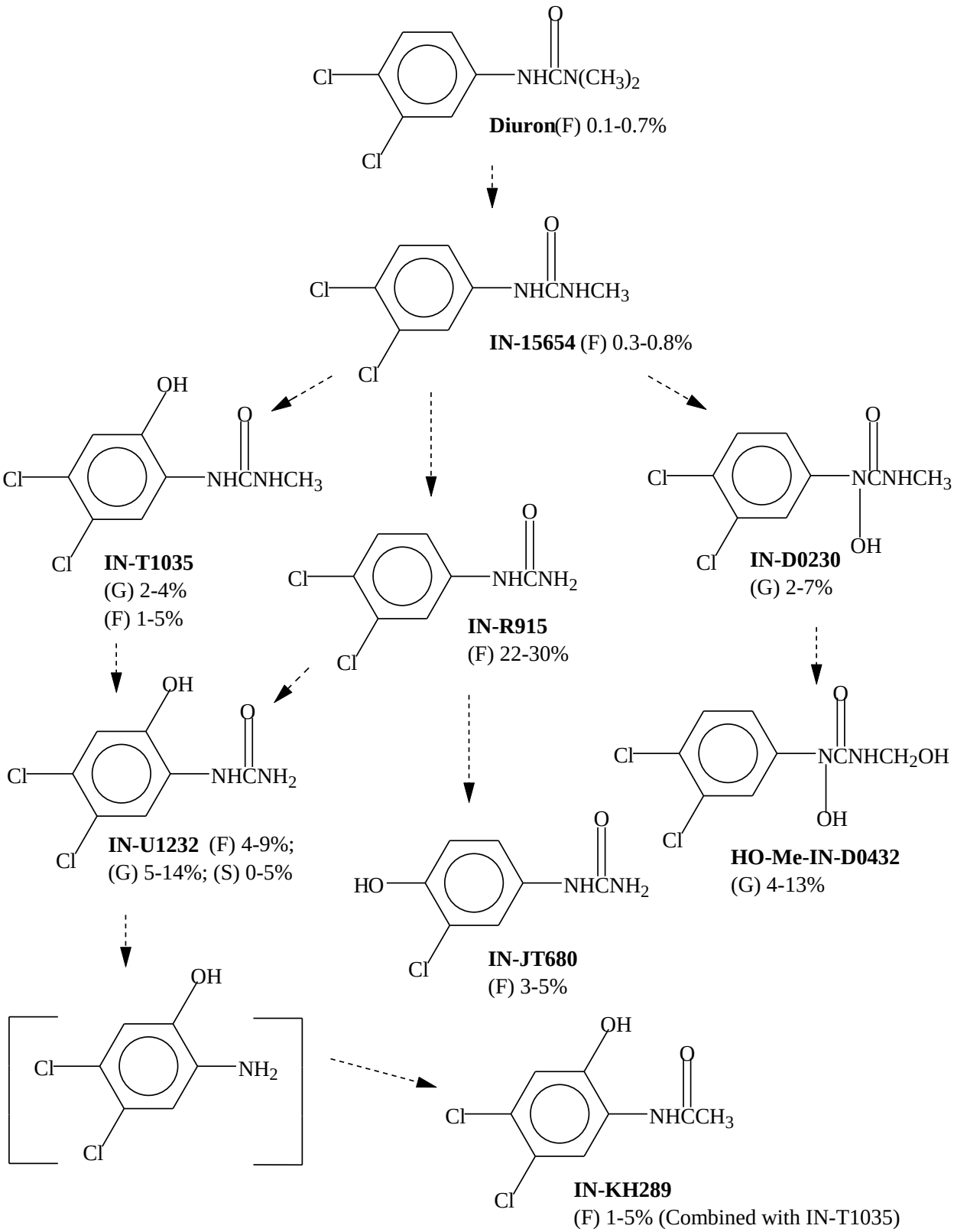
Distribution of identified diuron metabolites in combined excreta (%)

Code of Metabolites	Chemical Name of Metabolites	**10 mg/kg bw (single)**		**10 mg/kg bw (multiple)**		**400 mg/kg bw (single)**	
		M	F	M	F	M	F
IN-JT680	<N-(3-chloro-4-hydroxyphenyl)-urea>	4.76	2.91	5.29	3.45	4.24	2.73
Glu-HO-Me-In-D0432		5.91	7.85	3.55	8.12	9.65	13.4
Glu-IN-U1232		6.67	6.00	9.81	4.52	14.2	14.4
Glu-IN-D0230		3.86	6.77	1.94	6.99	6.45	7.43
Sulfate-IN-U1232		5.01	3.25	5.38	3.52	ND	ND
Glu-IN-T1035		3.00	3.26	3.23	2.31	3.02	3.89
IN-U1232	<N (4,5-dichloro-2-hydroxyphenyl)- urea>	6.82	7.17	6.03	8.61	4.97	6.33
IN-R915	<3-(3,4-dichlorophenyl)-urea>	28.5	29.3	30.3	26.2	24.2	22.0
IN-T1035/ IN-KH289	<3-(4,5-dichloro-2-hydroxyphenyl)-1-methyl urea /N-(4,5-dichloro-2-hydroxyphenyl)-acetamide>	2.57	3.46	2.36	4.76	1.17	1.75
IN-15654	<3-(3,4-dichlorophenyl)-1-methyl urea>	0.30	0.40	0.30	0.27	0.96	0.84
Diuron	<3-(3,4-dichlorophenyl)-1,1-dimethyl urea>	0.36	0.26	0.34	0.12	1.56	0.56
Urine Unknowns		19.8	16.8	21.5	19.8	15.2	10.3
Faeces Unknowns		2.60	2.21	2.64	2.05	3.63	3.25
Total		90.2	89.6	92.6	90.7	89.3	87.0

Metabolism of diuron was found to occur through a variety of pathways (see the following figure). Diuron was N-de-methylated to form IN-15654 and subjected to oxidative hydroxylation on both the aromatic ring (IN-T1035) and nitrogen atom (IN-D0230). IN-15654 underwent further N-demethylation to form IN-R915, the major excreted metabolite. IN-R915 was then hydroxylated to form IN-U1232, the second most predominant primary metabolite. N-hydroxylated products were excreted only in conjugated forms. All hydroxylated metabolites were conjugated primarily with glucoronic acid, except for IN-U1232, which was also conjugated with a sulfate moiety. The formation of IN-KH289, as a minor pathway, was probably through a 2-amino-4,5-dichlorophenol intermediate. Dechlorination of IN-R915 led to the formation of IN-JT680 as another minor metabolite. IN-D0230 (or its conjugate) could be further oxidized to form OH-Me-IN-D0432 glucuronide.

Proposed Metabolic Pathways of Diuron in Rats

See the Table above for key to codes.



Metabolite excreted in the free form (F), as a glucoronide (G), or as a sulfate (S).

It is noted from the inhalation study shown below (Pauluhn & Eben, 1986) that 3,4-dichloraniline in free and conjugated forms waw also detected in rat urine, suggesting alternative pathways which might not be included in the figure above.

Pauluhn J & Eben D-C A (1986). The concentration of diuron and its representative metabolites in the urine of male and female rats during a subacute inhalation study over eight weeks. Lab: Bayer AG, Fachbereich Toxikologie (Toxicology Unit) Report No. 14754.

Methods
Wistar rats (5/sex/dose) were exposed to 0, 4.1, 37.4 or 268 mg/m^3 of diuron aerosol in tubes (equivalent to head/nose exposure) for 6 h per day, 5 days per week for 8 weeks. After exposure for 4 or 8 weeks, the diuron concentrations in the aerosol were determined by HPLC. The urine samples were collected over 18 h for analysis of diuron and its metabolites before and after hydrolysis. Rats (Bor: WISW, SPF-Cpb), aged 8-10 weeks and 160-200 g, were from breeder Winkelmann Borchen, Kreis Paderborn. Ethanol : polyethylene glycol E 400 (1:1) was used as vehicle for production of diuron aerosol.

Results
The main metabolite excreted through urine was 3,4-dichlorophenyl urea that present solely in the free form. At least for the two high dose groups, females excreted less 3,4-dichlorophenyl urea than males. N-(3,4-dichlorophenyl)-N-methyl urea and 3,4-dichloraniline were mostly present in the conjugated forms in the urine extracts. Unchanged diuron was only detectable in the two high dose groups. A total of 11-19% of the dose (except female group at 37.4 mg/m^3) was excreted from the urine, as unchanged diuron, and as free or conjugated metabolites. The comparison of metabolite profiles between 4- and 8- week exposure did not reveal significant variations, and did not indicate accumulation of diuron or its metabolites.

Excretion of diuron and its metabolites in the 18-hour urine (□g/rat)

Dose (mg/m^3)	Total (mg / % dose)		Free Diuron		3-(3,4-dichlorophenyl)-1-methyl urea (free/conj.)		3-(3,4-dichlorophenyl) urea (free)		3,4-dichloroaniline (free/conj.)	
	M	F	M	F	M	F	M	F	M	F
4-week										
4.1	0.03 / 11.1	0.03 / 15.0	-	-	traces	Trace / 0.9	18.6	20.2	- / 6.2	- / 1.9
37.4	0.28 / 11.8	0.11 / 6.1	2.1	1.1	0.7 / 10	1.4 / 9.0	196	52.3	1.0 / 29	1.5 / 23
268	2.03 / 12.2	1.59 / 12.5	9.9	15.5	11 / 149	4.6 / 166	1282	819	19 / 252	23 / 299
8-week										
4.1	0.04 / 13.8	0.03 / 15	-	-	- / trace	- / trace	20.4	21.4	- / 3.0	- / 2.8
37.4	0.47 / 18.6	0.13 / 7.2	1.9	2.2	2.8 / 26	0.5 / 13	267	59.1	2.7 / 66	1.1 / 28
268	2.36 / 13.6	1.43 / 10.8	17.0	17.0	15 / 189	2.9 / 166	1367	605	25 / 371	29 / 348

The total includes diuron and its metabolites.

Due to limited reference substances available, the quantitative determination in the urine was limited to the above three metabolites.

Weber H & Abbink J (1988). [Phenyl-UL-^{14}C]-diuron: Investigation of the biokinetic behaviour in the rat. Lab: Institute for Metabolism Research, Metabolism Animal, PF-Zentrum Monheim, Building 6670, 5090 Leverkusen-Bayerwerk. Sponsor: Bayer AG/Sector 5, Business Group Agrochemicals, Research CE, Institute for Metabolism Research, Leverkusen / Monheim. Study No: M181068-4 & M 181069-5. Guidelines: US EPA 85-1 .

Methods

[Phenyl-UL-14C]-diuron was given to Wistar rats (5/sex/group) as a single iv dose of 5 mg/kg bw, or a single oral dose of 5 or 200 mg/kg bw, or oral doses of 5 or 200 mg/kg bw/day for 15 days (unlabelled doses for 14 days followed by a labelled dose). A group of 5 male rats with biliary fistulae was included. The excreta (urine, faeces) were collected for 72 h, and tissues/organs were collected at sacrifice 72 h post dosing. The radioactivity in the excreta, blood and tissues was measured by liquid scintillation. Since only a negligible amount (0.01%) of administered radioactivity was found in the expired air within 3 days after an oral dose of 200 mg/kg bw in preliminary test (2/sex), expired air was not analysed in the main study.

Results

For all doses, more than 92% of that administered was recovered, the majority of which was eliminated from the urine and faeces within 48 h. The excretion was mainly through the urine (66-92%), and to a lesser extent through the faeces (13-31%). A comparable amount of radioactivity excreted into the urine between the oral and iv dose groups indicated that the dose was completely absorbed following oral administration. An enterohepatic circulation was demonstrated by the group with biliary fistulae. The excretion profile following repeat dosing at low or higher levels, which was similar to the single dose groups, did not suggest accumulation.

Comparison of ^{14}C recovery from rat urine and faeces (%)

	5 mg/kg po, bile	**5 mg/kg, iv single dose**		**5 mg/kg, po single dose**		**5 mg/kg, po multi doses**		**200 mg/kg, po single dose**		**200 mg/kg, po multi doses**	
	M	M	F	M	F	M	F	M	F	M	F
Bile	35.8										
Urine#	54.5	89.8	81.7	76.4	92.4	81.3	84.0	65.6	78.6	86.6	86.1
Faeces	4.10	15.9	13.1	16.0	13.8	16.9	14.8	31.0	21.8	17.0	17.9
Body@	0.46	0.53	0.55	0.49	0.54	2.56	1.23	0.54	0.67	1.16	1.68
GIT	0.04	0.06	0.07	0.05	0.07	0.15	0.16	0.06	0.07	0.15	0.15
Total	92.3	106.3	95.5	92.9	106.8	100.9	100.2	97.1	101.1	104.9	105.9

#Urine includes cage rinse. @Body: all tissues tested excluding GIT.

At sacrifice 3 days post an oral dose, low residue levels (0.5-2.5% of the recovered amount) were found in the body excluding the GIT, i.e. corresponding to a mean body concentration of 0.027-0.031 g/g tissue after 5 mg/kg bw, and 1.18-1.34 g/g tissue after 200 mg/kg bw. These values increased by a factor of 3-5 after repeated dosing, suggesting a low bioaccumulation. The highest residual levels were measured in the erythrocytes, followed by kidneys, liver, spleen, adrenal gland, plasma and lungs. Lower residual levels were found in other tissues.

As shown in the following Table, the maximum relative plasma concentrations of (C_{max}) 0.29 to 0.58 were reached in a time range (T_{max}) of 1.70 to 6.80 h. The dose-corrected areas under the blood curves yielded an AUC 4.7-11.1 h. The terminal half-life $t_{1/2}$ was similar following either oral or iv dosing, single or repeat dosing, in the range of 26.2 to 34.4 h.

Biokinetic parameters form a model free blood curve analysis

	5 mg/kg, iv, single		**5 mg/kg, po, single**		**5 mg/kg, po, multi**	
	M	F	M	F	M	F
C maximum			0.58	0.57	0.37	0.29
T maximum (h)			1.88	1.70	5.60	6.80
AUC total (h)	9.56	11.12	4.69	5.91	9.35	8.94
$T_{1/2}$ (h) (terminal half-life)	27.8	32.1	34.3	28.3	26.2	30.6

3. ACUTE STUDIES

3.1 Technical Grade Active Constituent

Median Lethal Dose Studies

A summary of submitted and published findings of acute median lethal dose studies with technical diuron is shown in the Tables below.

Oral

Species [strain]	Sex	Group Size	Vehicle	Batch No / Purity (%)	Doses Tested (mg/kg bw)	LD50 (95% CI) (mg/kg bw)	Reference
Mice (NMRI)	M, F	8/S/D	25% tragacanth	- / ≥ 96%	0, 4700, 5400, 6200, 7100, 8130, 9330	M: 8590 (8483-8698) F: 8244 (7479-9088)	Kasa (1986)
Mice (ICR-JCL)	M, F	Not stated	0.5% tragacanth	- / 99%	Not stated	M: 1725 F: 1502	Du Pont (1980)
Rat (Crl: CD BR)	M	1/D	Corn oil	IN-15926-6 / 99.9	670, 2300, 3400, 5000, 7500, 11000	Death at 5000 and 11000	Sarver (1992b)
Rat (Crl: CD BR)	M	1/D	Corn oil	IN-12894-5 / 99.8	670, 2300, 3400, 5000, 7500, 11000	No death at 11000	Sarver (1992a)
Rat (SD)	M F	5/S/D	Propanol	292-03-93 / 98.5	2000	M: > 2000 F: > 2000 (all no death)	Wandrag (1993)
Rat	M	10-15/D	Water + cremophor	- / 97.3	2500, 3500, 5000	~ 5000	Kimmerle (1972)
Rat	M	10/D	Water + cremophor	- / 98.5	2500, 5000	> 5000	Thyssen (1974)
Rat	M	10/D	Water + cremophor	- / 99.4	3000, 5000, 10000	3000 - 5000	Thyssen (1975)
Rat	M	10/D	Water + cremophor	232114080/ 98.7	5000	> 5000	Mihail (1981)
Rat	M	10/D	unknown	232114156/ 98.5	1000, 2500, 5000	> 5000	Heimann (1981)
Rat (Wistar)	F	10/D	Water + cremophor	232114080/ 98.8	25, 50, 1000, 2500, 5000, 7100	4150 (2894-5951)	Heimann & Thyssen (1983)
Rat	M	5/D	Water + cremophor	232114123 / -	3000, 3550, 4000, 5000	4138 (2866-5978)	Heimann (1984)
Rat	M F	10/D	Peanut oil	Not stated	Not stated	M: 1258 (999-1583); F: 1182 (995-1450)	Gaines & Linder (1986)
Rat (Wistar)	M F	8-10 /S/D	25% tragacanth	- / ≥ 96%	0, 4700, 6200, 7100, 8130, 10000	M: 9087 F: 6894	Kasa (1986)
Rat (albino)	M	10-15 /D	Cotton seed oil	- / 95%	Not stated	1017 (795 – 1239)	Boyd & Krupa (1970)
Rat (Wistar)	M F	5/S/D	Peanut oil	D9SP074 / 97%	2300, 3250, 4600	3089 (2415-3952)	Rana (1999)

Abbreviations: NS = not specified; SD = Sprague-Dawley; M = male; F = female; /S/D= /sex/dose; Es = estimated.

Dermal

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Rat (SD)	M F	5/S/D	Propanol	292-03-93 / 98.5	2000 (24 h, covered)	M: > 2000 F: > 2000 (no deaths)	Wandrag (1993)
Rat (Wistar)	M F	5/S/D	Saline	232114080/ 98.8	2500, 5000,	> 5000 (no death)	Heimann & Thyssen (1983)
Rat	M F	10/D	Propylene glycol	Not stated	Not stated	> 2500	Gaines & Linder (1986) (no data)
Rat (Wistar)	M F	8-10 /S/D	25% tragacanth	Diuron technical active (no details)	0, 2000	> 2000 (no deaths)	Kasa (1986)
Rats (Wistar)	M F	5/S/D	Peanut oil	D9SP074 / 97%	0, 2000	> 2000 (no deaths)	Rana (1999)

Abbreviations: NZW=New Zealand White; DMSO: dimethyl sulfoxide.

Inhalational

Species [strain]	Sex	Group Size	Vehicle/ mode	Purity (%)	Concentrations Tested (mg/m^3)	LC50 (mg/m^3)	Reference
Rat [Crl:SD BR]	M F	10/S/D	Dust /nose only/4h	IN-14740-146 / 99.0	0, 7000, 7100 (MMAD > 10 µm or ~ 10.3 µm)	> 7100 (M/F), (no deaths)	Kinney (1987)
Rat (Wistar)	M F	10/S/D	Ethanol-lutrol/4h	232114080/ 98.8	73, 195, 223	> 223 (no deaths)	Heimann & Thyssen (1983)
Rat (Wistar)	M F	10/S/D	Dust/whole body /4h	Diuron tech (no details)	6200 (50% < 7 µm)	> 6200 (no deaths)	Beres (1986)
Rat (Wistar)	M F	9/S/D	Dust/whole body /4h	D9SPO74 / 97%	2139 (MMAD 6.16 µm)	> 2139 (no deaths)	Joshi (2000)

Abbreviations: SD=Sprague-Dawley.

3.1.1 Oral

Kasa Z (1986). Study on the acute toxicity of diuron technical active ingredient Vol II (Acute oral toxicity in mice). Lab: NEVIKI Research Institute for Heavy Chemical Industries. Guidelines: not stated. Veszprem.

Fasted mice (8/sex/dose) received an oral dose of diuron at 0, 4700, 5400, 6200, 7100, 8130 or 9330 mg/kg bw. Males at 8130 mg/kg bw and above showed lower body weight gain. Ruffled fur, decreased motility, low responses to exogenous triggers, mild dyspnoea, somnolence, chemosis/blood tears, humped posture, weakness of the extremities, decreased grasping reflex, lack of response to sound and pain triggers were observed from 1 h after the treatment. Deaths occurred in 1, 1, 2 and 4 males and 1, 1, 4 and 6 females respectively at 6200, 7100, 8130 and 9330 mg/kg bw within 2 days. Autopsy revealed damaged mucosa, and thin and transparent gastric wall in the stomach, markedly hyperaemic mucosa in the duodenal portion of the small intestine, and darker spleen with black nuance. The general state of surviving mice gradually improved. Mice at 8130 and 8330 mg/kg bw still showed toxic symptoms on Days 5-6, but fully recovered after Day 7. Darker and enlarged spleen was

also seen in these mice at the study termination. A Probit analysis yielded a LD50 8590 (8483 – 8698, at 95% probability level) mg/kg bw for males and 8244 (7479 - 9088) mg/kg bw for females.

Wandrag S (1993). Oral and Dermal limit test with Sanachem Sanuron (Diuron technical) in rats. Batch no: 292-03-93. Report No: 1. Lab: Roodeplaat Research Laboratories (Pty). Sponsor: Sanachem (Pty) Ltd. Ltd. Report date: 2/7/1993. GLP/QA: Yes. Guidelines: OECD 401&402.

A single dose of 2000 mg/kg bw of diuron was given to fasted rats (5/sex) by gavage. There were not deaths. Animals were found to be lethargic 2 h after dosing, but recovered later of the day. No more signs were observed during the 14-day observation period, and no macroscopic pathological changes were found in necropsy on Day 14. The oral LD50 was greater than 2000 mg/kg bw.

Sarver JW (1992). Approximate lethal dose (ALD) of IN-12894-5 in rats. Medical Research No: 4581-951. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware. Sponsor: Du Pont Agricultural Products, Wilmington, Delaware. Study Completion date: 10/2/1992. GLP/QA: Yes. Guidelines: None.

A single dose of diuron at 670, 2300, 3400, 5000, 7500 or 11000 mg/kg bw was given by intragastric intubation to male rats (1/dose). During the 14-day observation period, animals at all dose levels showed weight loss (up to 13%), lethargic, low posture, dry red ocular/red ocular discharge, nasal and oral discharges, and/or wet/yellow-stained perineum. There were no deaths at any doses. The LD50 can not be determined due to only a single animal at each dose level.

Sarver JW (1992). Approximate lethal dose (ALD) of IN-12894-6 in rats. Medical Research No: 4581-952. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware. Sponsor: Du Pont Agricultural Products, Wilmington, Delaware. Study Completion date: 12/3/1992. GLP/QA: Yes. Guidelines: None.

A single dose of diuron at 670, 2300, 3400, 5000, 7500 or 11000 mg/kg bw was given by intragastric intubation to male rats (1/dose). During the 14-day observation period, animals at 2300 mg/kg bw and higher doses showed weight loss, lethargic, low posture, dry red ocular discharge. The rats at 5000 and 11000 mg/kg were found dead on day 2 and day 4 respectively. The LD50 can not be determined due to only a single animal at each dose level.

Heimann KG & Thyssen J (1983). Diuron active ingredient studies on acute toxicity. Lab: Bayer AG Institute for Toxicology. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld Germany. Report No: 11710. Study Duration: September 1982 – January 1983. GPL: no.

Fasted female rats (10/dose) received a single dose of diuron at 25, 50, 1000, 2500, 5000 or 7100 mg/kg bw by gavage. Rats at 50 mg/kg bw and higher showed behaviour, respiratory and motility disorders, staggering, spastic gait, lying in side position or on stomach, and/or narcosis-like state within 30 min to 2 h after dosing, with some signs lasting for 12 days. Mortality occurred in 3, 5 and 9 rats at 2500, 5000 and 7100 mg/kg bw respectively, and necropsy revealed distended lungs, patchy in lungs, liver, kidneys and spleen, glandular and reddened stomach, and/or dark spleen in these rats. Slightly enlarged and dark spleen was also observed in rats surviving to the study termination. The oral LD50 was 4150 (2894 – 5951) mg/kg bw.

Female rats (10/dose) received an intraperitoneal injection of diuron at 25, 50, 250, 315, 400 or 500 mg/kg bw. Clinical signs including behaviour, respiratory and motility disorders, staggering, spastic gait, lying in side position or on stomach, and/or narcosis-like state occurred at 50 mg/kg bw or higher doses within 2 h and some lasted for 10 days. 2, 7 and 9 rats died at 315, 400 and 500 mg/kg bw respectively and necropsy revealed distended lungs, patchy in lungs, liver, kidneys and spleen, and/or dark spleen in these rats. The LD50 was 368 (317-427) mg/kg bw.

Kasa Z (1986). Study on the acute toxicity of diuron technical active ingredient Vol I. (Acute oral toxicity in rat, acute percutaneous toxicity in rat, acute intra-peritoneal toxicity in rat, acute eye irritant effect in rabbit, acute dermal irritant effect in rabbit). Lab: NEVIKI Research Institute for Heavy Chemical Industries. Veszprem.

Fasted rats (8-10/sex) were given a dose of diuron at 6200/4700 (M/F), 7100, 8130 or 10000 mg/kg bw by gavage. Signs including vigourless movement, uncoordinated, rapid breathing, hardly response to exogenous triggers, dyspnoea, ruffled fur, mild salivation, hyperaemic eyes and nasal discharge were observed at all dose groups within 4 h. The general state of survivors was improved after 24 h, and fully recovered by Days 5-6. Lower body weight was seen in all treated male groups in Week 1, and in female groups at 6200 mg/kg bw and above. Death occurred in 0/8, 1/8, 2/8 and 7/10 males at 6200, 7100, 8130 and 10000 mg/kg bw, and in 3/8, 3/8, 5/8 and 7/10 females at 4700, 7100, 8130 and 10000 mg/kg bw during Days 1-2. Findings on these rats at necropsy included dark spleen, and dilated stomach with several erosion spots in the pylorus and filled with the yellow-white test chemical. The LD50s were determined at 9087 and 6894 mg/kg bw for males and females.

Fasted rats (8/sex/dose) was given a dose of diuron at 0, 420, 510, 620, 760 or 940 mg/kg bw in the form of 25% tragacanth suspension by intraperitoneal injection. Signs including increased respiratory rate, ruffled fur, reduced motility, brownish nasal discharge, no response to exogenous triggers, progressing dyspnoea and comatose state develop at all dose levels within 3 h, indicating central disturbances of the circulation and respiration. The state of survivors improved after 10 h, and fully recovered by day 4. Lower body weights were observed for males and females during the first week. Deaths occurred in 0, 1, 2, 3 and 6 males, and 0, 3, 4, 7, 6 females at 420, 510, 620, 760 and 940 mg/kg bw respectively within 8-25 h. Necropsy revealed lungs with congestive hyperemia, heart with passive hyperemia, thymus with pinpoint haemorrhage, dark brown liver with yellow-white spots, mucosa of stomach with pyloric erosion, ileum with haemorrhage infiltration, kidneys in apparent anaemia, and dilated urinary bladder in these animals. The LD50s were determined at 786 (666 – 926) and 627 (542 – 726) mg/kg bw for males and females.

Rana MD (1999). Acute oral toxicity study of diuron technical 97% in rats. Lab: Department of Toxicology, JAI Research Foundation, Valvada – 396 108 Dist. Valsad, Gujarat, India. Guidelines: EPA Series 81-1. GLP/QA: Yes.

Rats (5/sex/dose) received a single dose of diuron at 0, 2300, 3250 or 4600 mg/kg bw by gavage. Clinical signs including lethargy, toe walking, abdominal breathing, nostril discharge, lacrimation and/or salivation were observed in all treated groups in the first 2 days, and disappear thereafter. Deaths occurred in 1, 3 and 5 males, and 2, 2 and 3 females at 2300, 3250 and 4600 mg/kg bw respectively within 2 days post-dosing. Pathology revealed mottling in liver, and catarrhal inflammation in duodenum as well as other intestine segments of dead and surviving rats at termination. The LD50 was 3089 (2415 – 3952) mg/kg bw for males and females in combination.

Boyd D & Krupa V (1970). Protein-deficient diet and diuron toxicity. J Agr Food Chem. 18:1104-7. Report No: not stated. GLP: no.

Male albino rats fed from weaning for 28 days with (1) a protein-deficient diet containing 3.5% casein; (2) a normal protein diet containing 26% casein or (3) a standard laboratory chow. Body weights of the three groups were 58, 202 and 172 g respectively at the end of feeding period. The acute oral toxicity of diuron (suspended in cottonseed oil. Purity: 95%, from Industrial and Biochemicals Department, El du Pont de Nemours, Wilmington, Del) was then measured in fasted rats (10-15/dose, doses used were not specified) of these groups. Deaths occurred within 2 days after dosing. Gross pathology revealed gastritis, enteritis and dehydrated caecum in all three groups, as well as congested brain and lungs, and yellowish kidneys in Groups 2 and 3. The LD50 values were 437 $\pm$139, 2390 $\pm$ 1440 and 1017 $\pm$ 222 mg/kg bw respectively for the 3 groups, suggesting that protein-deficiency might enhance the acute toxicity of diuron.

3.1.2 Dermal

Wandrag S (1993). Oral and Dermal limit test with Sanachem Sanuron (Diuron technical) in rats. Batch: 292-03-93. Report No: 1. Lab: Roodeplaat Research Laboratories (Pty). Sponsor: Sanachem (Pty) Ltd. Ltd. Report date: 2/7/1993. GLP/QA: Yes. Guidelines: OECD 401&402.

Sprague Dawley rats (5/sex) received a dermal application of diuron at 2000 mg/kg bw as a paste to the clipped dorsal and ventral area of the trunk (1/10 body surface) for 24 h. There were on deaths. No symptoms or macroscopic pathology were observed. The dermal LD50 was greater than 2000 mg/kg bw.

Heimann KG & Thyssen J (1983). Diuron active ingredient studies on acute toxicity. Lab: Bayer AG Institute for Toxicology. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld Germany. Report No: 11710. Study Duration: September 1982 – January 1983. GPL: no.

Rats (5/sex/dose) received a dermal application of diuron 2500 or 5000 mg/kg bw on the shaved dorsal skin for 24 h. Reduced motility and apathy developed at 5000 mg/kg bw 1 day after the application, and remained for 2-4 days. No animals died during the 14-day observation, and no macroscopic lesions were found either on the skin or in any other organs at termination. The dermal LD50 was greater than 5000 mg/kg bw.

Rana MD (1999). Acute dermal toxicity study of diuron technical 97% in rats. Lab: Department of Toxicology, JAI Research Foundation, Valvada – 396 108 Dist. Valsad, Gujarat, India. Guidelines: EPA Series 81-2. GLP/QA: yes.

Rats (5/sex/dose) received a dermal dose of diuron at 0 or 2000 mg/kg bw on pre-clipped intact skin of the dorsal area of the trunk for 24 h. There were no deaths, no clinical signs or treatment-related pathological alterations. The LD50 was greater than 2000 mg/kg bw.

Kasa Z (1986). Study on the acute toxicity of diuron technical active ingredient Vol I. (Acute oral toxicity in rat, acute percutaneous toxicity in rat, acute intra-peritoneal toxicity in rat, acute eye irritant effect in rabbit, acute dermal irritant effect in rabbit). Lab: NEVIKI Research Institute for Heavy Chemical Industries. Veszprem. Guidelines: not stated. GLP/QA: not stated.

Rats (10/sex/dose) was given a dermal application of diuron 2000 mg/kg bw onto a 64 cm^2 skin area. There were no deaths and no any treatment-related findings. The LD50 was greater than 2000 mg/kg bw.

3.1.3 Inhalation

Kinney LA (1987). Acute inhalation toxicity study with diuron in rats. Code: IN-14740-146. Lab/Sponsor: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware / Du Pont Agricultural Products, Wilmington, Delaware. Study Completion date: 20/3/1987. Medical Research No: 4581-432. GLP/QA: yes. Guidelines: US EPA 81-3.

Wistar rats (10/sex/dose) were exposed nose-only to dust atmospheres of diuron at 0, 7000 or 7100 mg/m^3 for 4 h. There were no deaths. Red nasal discharge, lethargic and partially closed eyes were observed during exposure, and these signs together with hair loss, red ocular/oral discharges, stained perineum, and stained or discoloured fur and weight loss occurred in some treated rats during post exposure period. On Day 14, weight gain was comparable between groups, and no gross abnormalities were detected at necropsy. The LC50 was greater than 7100 mg/m^3 in this study.

Heimann KG & Thyssen J (1983). Diuron active ingredient studies on acute toxicity. Lab: Bayer AG Institute for Toxicology. Lab/Sponsor: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld Germany. Report No: 11710. Study Duration: September 1982 – January 1983. GPL: no.

Wistar rats (10/sex/dose) inhalated aerosol of diuron at 73, 195 or 233 mg/m^3 (formulated in an ethanol-lutrol mixture 1:1 to a concentration of 0.5-7.5%) with tidal air once for 4 h, or at 21, 77 or 260 mg/m^3 for 6 h x 5 days. The rats of the single dose groups displayed non-specific behaviour disorders up to 2 h post-exposure, with no deaths and no organ lesions at necropsy. The inhalation LC50 was greater than 233 mg/m^3.

For the repeated exposure groups, rats at 260 mg/m^3 exhibited slightly pronounced non-specific behaviour disorders during the first 3 days of exposure, and lost body weight slightly. There were no deaths in the study.

Beres E (1986). Acute inhalation toxicity test of diuron technical active ingredient on Wistar rat. Lab: NEVIKI Research Institute for Heavy Chemical Industries. Veszprem. GLP/QA: not stated. Guidelines: not stated.
Wistar rats (9/sex/dose) were exposed to the dust of 0 or 6200 mg/m^3 of diuron for 4 h. Only 50% of the test substance produced in the dynamic inhalation system fell into the respirable range (< 7 □m). There were no deaths. Blood discharge in the nose/eyes, balance disorders, ataxic motion and decreased motility were observed in the treated group, and lower body weight in treated males. No macroscopic changes were seen at necropsy. The LD50 was greater than 6200 mg/m^3.

Joshi G (2000). Acute inhalation toxicity study of diuron technical 97% in rats. Lab: Department of Toxicology, JAI Research Foundation, Valvada – 396 108 Dist. Valsad, Gujarat India. Guidelines: EPA Series 81-3. GLP/QA: yes.

Wistar rats (10/sex/dose) were exposed to a dust of 0 or 2139 mg/m^3 of diuron for 4 h. The MMAD was 6.16 □m, with 58% of particles generated ≤ 4.45 □m. The exposure did not cause death or clinical signs. Diffused pneumonic foci or emphysema was seen in 3 treated rats at necropsy. The LD50 was greater than 2139 mg/m^3.

3.1.4 Skin Irritation Studies

Sharp VW (1997b) Acute dermal irritation/corrosion test with Sanachem diuron technical in rabbits. Lab: Biocon Research Pty Ltd, Pretoria 0001, South Africa. Report No: 1281. Guidelines: OECD 404. . GLP/QA: yes.

Diuron 500 mg (Batch No: 212/02/96, Purity: 99.20%) was applied to one intact skin site (6 cm^2) of a clipped dorsal area in the trunk of 3 female NZW rabbits for 4 h. Skin response was graded and scored at 1, 24, 48 and 72 h post-exposure according to Draize scale. No erythema or oedema was observed at any application sites (all score 0). The test material was not a skin irritant in rabbits.

Bakili RA (2000). Primary dermal irritation study of diuron technical 97% in rabbits. Lab: Department of Toxicology, JAI Research Foundation, Valvada – 396 108 Dist. Valsad, Gujarat India. Guidelines: EPA Series 81-5. GLP/QA: Yes.

Rabbits (3/sex) received dermal applications of 0 and 500 mg of diuron (Batch No: D9SP074, Purity: 97%, peanut oil as vehicle) on contralateral two sites of preclipped intact skin of the dorso-lumbar region for 4 h. The sites were examined at 1, 24, 48 and 72 h post-exposure and scored according to the Draize method. No skin reactions were observed at any time (all score 0). The test substance was not a skin irritant.

Kasa Z (1986). Study on the acute toxicity of diuron technical active ingredient Vol I. (Acute oral toxicity in rat, acute percutaneous toxicity in rat, acute intra-peritoneal toxicity in rat, acute eye irritant effect in rabbit, acute dermal irritant effect in rabbit). NEVIKI Research Institute for Heavy Chemical Industries. Veszprem. Guidelines: not stated. GLP/QA: not stated.

Six NZW rabbits received an application of 500 mg diuron on both the intact and scarified skin surface of the trunk which was pre- depilated with sodium sulphide and neutralised with acetic acid. The area was covered with a moistened sheet of gauze and folio and fixed with a plaster. Skin irritation was evaluated at 24, 48, 72 and 168 h as well as 2 weeks later. Mild erythema (score 1) was seen in 2/6 intact skin sites, and in 3/6 scarified skin sites at 24 h, but disappeared after 2-days. Parchment-like skin necrosis (score 1 or 2) occurred in 3/6 intact skin sites, and in all scarified skin sites followed by peeled off within 3-5 days. The skin recovered completely from all symptoms within 7 days. The test substance was a moderate skin irritant.

Du Pont (1980). Toxicological information. Report No: 51578.

No irritation was observed following a single dose (0.05 g or 50% aqueous) of diuron (81-97% active was applied to intact skin occluded and contacted for 3-6 days.

3.1.5 Eye Irritation Studies

Sharp VW (1997a). Acute eye irritation/corrosion test with Sanachem diuron technical in rabbits. Lab: Biocon Research Pty Ltd, Pretoria 0001, South Africa. Report no: 5/07/1903. Guidelines: OECD 405. GLP/QA: yes.

Diuron TC powder 0.1 g (Batch No: 212/02/96, Purity: 99.20%) was instilled into the conjunctival sac of the left eye of 3 female NZW rabbits. Irritation was graded and scored at 1, 24, 48 and 72 h according to Draize method. Slight erythema and conjunctival chemosis (score 1-2) occurred in all 3 treated eyes at 1 and 24 h, and slight erythema was seen in 1/3 eyes at 48 h. The test substance was a slight eye irritant.

Bakili RA (2000). Primary eye irritation study of diuron technical 97% in rabbits. Lab: Department of Toxicology, JAI Research Foundation, Valvada – 396 108 Dist. Valsad, Gujarat India. Guidelines: EPA Series 81-4. GLP/QA: Yes.

Six male NZW rabbits received an instillation of 100 mg of diuron (Batch No: D9SP074, Purity: 97%) into one eye while the contralateral eye served as a control. Observations for changes in the conjunctiva, iris and cornea (using fluorescein staining at 24 h) were made at 1, 24, 48 and 72 h. Slight redness (score 1) was observed in 5/6 treated eyes at 1 h, and partial erosion (1/4 area) of corneal epithelium was in 2/6 treated eyes at 24 h, and no reactions of irritation was observed at 48 and 72 h. The test substance was a slight eye irritant.

Kasa Z (1986). Study on the acute toxicity of diuron technical active ingredient Vol I. (Acute oral toxicity in rat, acute percutaneous toxicity in rat, acute intra-peritoneal toxicity in rat, acute eye irritant effect in rabbit, acute dermal irritant effect in rabbit). Lab: NEVIKI Research Institute for Heavy Chemical Industries. Veszprem.

Nine NZW rabbits received 50 mg of diuron in the conjunctiva sack of one eye. Five minutes later, the treated eye of 3 rabbits was rinsed with cool water for 1 min, and others remained unrinsed. Eye irritation was evaluated according to the scoring system of Draize at 24, 48, 72 and 168 h after treatment, as well as 2 weeks later. Hyperaemia of the conjunctive developed immediately after dosing (no score was recorded), and disappeared within 24 h (score 0). The test substance was not considered to be an eye irritant.

3.1.6 Skin Sensitisation Studies

Sharp VW (1997c). Contact hypersensitivity to Sanachem Diuron Technical in albino guinea pigs (Magnusson Kligman Maximisation Test). Lab: Biocon Research Pty Ltd, Pretoria 0001, South Africa. Report no: 1283. Guidelines: OECD 406. GLP/QA: Yes.

The sensitisation of diuron technical (Batch No: 212/02/96, Purity: 99.20%) was tested in a test group of 20 and a control group of 10 female guinea pigs. On Day 1, 3 pairs of intradermal injections were made in a skin area of clipped flanks: (1) Freund's complete adjuvant with physiological saline; (2) 10% diuron/physiological saline (for test/control group); and (3) Mixture of 1 & 2. On Days 6-8, 50% diuron/saline was epidermally applied to the above area of the test/control animals for 48 h under dressing. On Days 20-22, 50% diuron was applied to the skin of clipped area of flank for 24 h under dressing. The concentrations of diuron were chosen based on a pre test. Observations for skin responses were made at 24 and 48 h after the exposure. No erythema or oedema was observed in any animals at any time (all score 0). The test substance was not a skin sensitiser.

Henry (1987). Dermal sensitisation study with IN-14,740-146 in guinea pigs. Lab: El du Pont de Nemours and Company, Inc., Heskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, PO Box 50, Newark, Delaware 19714. Report No: HLR 640-86. Guidelines: US EPA 81-6. GLP/QA: yes.

Methods
During the induction phase, sacral intradermal injections of 0.1 mL of 1.0% diuron (Purity: 99%, in dimethyl phthalate) were made into shaved intact shoulder skin of 10 male guinea pigs in the test group, or a 1.0% p-phenylenediamine to 10 males of the positive control group, once a week for 4

weeks. After 2 weeks, the test animals were challenged by applying and lightly rubbing in 1 drop of 80% and 8% diuron onto separate sites of skin, and the positive control animal by a drop of 30% and 3% p-phenylenediamine. Ten guinea pigs in a concurrent negative control group also received a dermal application of the challenge dose of diuron. Responses were scored 24 h after each induction application, and 24 and 48 h after challenge. The same groups also received a primary irritation test 2 days before the induction, and neither the test substance nor the positive control at challenge concentrations induced any skin responses.

Results
Following each intradermal injection with either diuron or the positive control during the induction, all animals exhibited erythema, oedema, blanching and necrotic centre (score 1 to 4), with responses more severe in the test animals than the positive control animals. While significant enhancement in skin responses was observed in positive control animals after challenge, the dermal application with 80% diuron (but not 8%) at challenge produced only mild erythema (score 1) in 3/10 test animals at 24 and 48 h. There were no skin responses in the negative control group. Since no significant increase in the sensitisation score was induced by the test substance, it was not considered to be a skin sensitiser.

Kasa Z (1986). Acute skin irritation and sensitisation testing of diuron technical active ingredient on guinea pigs. Lab: NEVIKI Research Institute for Heavy Chemical Industries. Veszprem. Guidelines: not stated.

In a preliminary skin irritation test, 3 albino guinea-pigs received a dermal application of 500 mg of diuron (purity not stated) on shaved skin (2x2 cm) of the right side of the trunk for 23 h. The skin irritation was evaluated at 24, 48, 72 and 144 h. The test substance did not show dermal irritant in guinea pigs.

In the sensitisation test, guinea pigs (3/sex) received dermal applications of 50 mg test substance on the pre shaved skin of the trunk for 6 h, alternatively at the right or left side, for 3 times per week and a total of 10 applications. The skin responses were evaluated at 24 h after each application. 14 days after the last application (Day 35), a provoking (challenging) dermal treatment (50 mg) was performed. The same provoking procedure was also performed on a control group of 3 guinea pigs without pre-treatment. The skin sensitizing effect was evaluated 24 h later. The sensitising applications did not cause any detectable skin lesion, and the response to the provoking treatment was also negative (all score 0). The test substance was non-allergenic in guinea pigs.

Bakili RA (1999). Dermal sensitisation study of diuron technical 97% in guinea pigs [Buehler test). Lab: Department of Toxicology, JAI Research Foundation, Valvada – 396 108 Dist. Valsad, Gujarat India. Guidelines: EPA Series 81-6. GLP/QA: yes.

During the sensitisation phase, Hartley guinea pigs (10/sex for the treated group and 5/sex for the control) received 0 or 500 mg of diuron (Batch No: D9SP074, Purity: 97%, moistened with peanut oil) on the clipped area of the left flank for 6 h under dressing, once per week for 3 weeks. On day 28, the animals were exposed to a challenge exposure of 500 mg of diuron on the right frank for 6 h. The skin reactions were observed at 24, 48 and 72 h after the challenge exposure, and graded to the Magnusson and Kligman grading scale. The reliability of the test system was proved by using p-phenylenediamine as the positive control. No dermal reactions were observed in any animal during the challenge phase (all score 0). The test substance was not a skin sensitiser under the test condition.

4. SHORT-TERM REPEAT-DOSE STUDIES

4.1 Oral Administration

(no data).

4.2 Dermal Application

McKenzie S A (1992). Repeated dose dermal toxicity: 21-day study with DPX-14740-166 (Diuron) in rabbits (Revision No 1). Lab/Sponsor: Du Pont Agricultural Products, E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report-no. HLR 484-92. Medical Research No: 9122-001. Study completion: 17/9/1992. GLP/QA: yes. Guidelines: US EPA F82-2; OECD 410.

Methods
New Zealand White rabbits (5/sex /dose) received dermal applications of diuron at 0, 50, 500 or 1200 mg/kg bw/day on shaved intact skin (approximate 190 cm^2 or 10% of the total body surface) from the scapular to the lumbar region of the back under cover for 6 h per day, 21 consecutive days. Doses were determined by a range finding study. Each dose of the test substance (Lot 2507, Purity: 96.8%, supplied by DuPont Agricultural Products, EI du Pont de Nemours and Company, Wilmington, Delaware) was mixed with deionised water before application. Rabbits were received from Hazleton Research Products, Denver PA, and were approximately 17 weeks of age, and 1814-2934 g on the first day of dosing.

Clinical observation was made daily, body weight measured twice per week and food consumption weekly. Blood samples were collected before dosing and at termination for haematology (Appendix VI, excluding clotting parameters and blood smear) and clinical chemistry (Appendix VI, excluding GGT, LDH, triglycerides, CPK). All rabbits were killed by barbiturate anaesthesia at necropsy on Day 22/23. All organs/tissues were grossly checked, selected organs (adrenals, liver, kidneys and testes) were weighed, and all organs/tissues (Appendix VI) from 0 and 1200 mg/kg bw/day groups and selected organs (treated and untreated skin, liver, gall bladder, kidneys and gross lesions) from 50 and 500 mg/kg bw/day groups were examined histopathologically.

Results
No deaths occurred during the study. Although slight to mild erythema was seen in all groups including control which was probably due to daily washing and bandaging, a higher incidence of mild erythema occurred in treated groups, in particular, moderate erythema and/or slight to mild oedema in 3 rabbits at 1200 mg/kg bw/day. Dermatitis in treated and/or untreated skin and a few clinical signs were observed in some animals across all groups, without a relationship with the treatment and dose levels. Animals in almost all groups including the control lost weight intermittently, and this may be related to the stress of daily handling. No biologically significant alterations were observed in haematologic or clinical chemistry parameters, mean organ weights, gross- or histo-pathology at any dose levels.

The NOEL for systemic changes was 1200 mg/kg bw/day, the highest dose tested.

Mihail D-BF & Schilde B (1984). Diuron subacute dermal toxicity study on rabbits. Lab: Institute of Toxicology, Bayer AG, Wuppertal-Elberfeld.. Report-no. 12360. Study No: T3016060. Study Duration: June – July 1983.

Methods
New Zealand White rabbits (6/sex /dose) received dermal applications of diuron at 0, 50 or 250 mg/kg bw/day on intact or abraded skin of the dorsal and flank (approximate 6 x 9 cm in size) under cover for 6 hours per day, 5 days per week for 3 weeks (a total of 15 day-treatment). The rabbits were received kg from Breader Hacking & Churchill, Huntingdon, England, and were 2.4-3.0 kg at the beginning of the study. The test substance (Batch: 232114080, Purity: 98.8%) was formulated with Cremophor EL in deionised water before application.

Results No deaths occurred during the study. Slight, transient erythema appeared on the treated intact skin site of 1 rabbit at 250 mg/kg bw/day in Week 2, and slightly more severe and longer lasting skin responses (erythema, skinfold thickness) were observed on the abraded skin site of some treated rabbits. There were no treatment-related findings in haematology, clinical chemistry and urinalysis. No abnormal observations were revealed in organ weights, gross and histo-pathology.

The NOEL for systemic effects was 250 mg/kg bw/day, the highest dose in this study.

4.3. Inhalational Administration

Pauluhn J (1986a). Diuron: Study for subacute inhalation toxicity to the rat (aerosol exposure 15 x 6 hours). Lab: Bayer AG, Institute for Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-233, West Germany. Report No: 14696. GPL: yes. Guidelines: OECD 412.

Methods
Wistar rats (10/sex/dose) were exposed head/nose only to the air, the solvent (lutrol/ethanol), 6.6, 48 or 311 mg/m^3 of diuron (Batch No: 232114080, Purity: 98.9%) aerosol for 6 h per day, 5 days per week for 3 weeks (15 x 6 h). The rats obtained from Breeder Winkel-Mann, Borchen, Kreis Paderborn, feed with Altromin 1324 Diet and were 160 – 200 g at the beginning of the study. The solvent, Lu/EtOH aerosol up to a nominal 20 mL/m^3, was tolerated by rats without undesirable effects. The aerosol concentrations of diuron and particle size (MMAD: approximate 2.0 □m with 92% ≤ 5 □m) were determined by sample analysis.

Body weights were determined weekly. Blood samples were collected by heart puncture at necropsy for examinations of haematology (see Appendix V, including Heinz bodies,) and clinical chemistry (glucose, urea, bilirubin, creatinine, total protein, AP, ALT, AST, GLDH, TBK, T3 & T4), and urine samples were collected for urinalyses (blood, protein, glucose, pH, sediment, ketone, bilirubin, urobilinogen). Cytochrome P-450, N-demethylase and O-demethylase activities in the liver were measured. Selected organs (adrenals, heart, kidneys, liver, lungs, spleen, thyroid, testes and ovaries) were weighed, all organs/tissues were grossly checked, and selected tissues (eyes, femur and sternum, lung hilus lymph nodes, heart, testicles/ovaries, head, liver, lungs, stomach, spleen, adrenals, kidneys, oesophagus, trachea with larynx and thyroids) were histopathologically examined. It was stated by the study author that investigation of potential urinary bladder alterations was not a subject of this study.

Results
All rats survived. Rats at 311 mg/m^3 showed piloerection during Week 3 of the exposure period, and males of this group had lower body weight gains.

Both sexes at 311 mg/m^3 and females at 48 mg/m^3 exhibited concentration-related Heinz body formation, MCV and MCH increases with simultaneous reticulocytosis, as well as a slight fall in the erythrocyte count. The increase in MCV at 311 mg/m^3 indicated reduced stability and hence shortened erythrocyte life, and other alterations were attributed to secondary heightened erythropoiesis. A significant increase in Heinz body of females at 6.6 mg/m^3 was dismissed since there were no related changes in other haematological parameters, as well as in other sex.

Changes in haematology

Concentration (mg/m^3)	**male**					**female**				
	Air	**Solv**	**6.6**	**48**	**311**	**Air**	**Solv**	**6.6**	**48**	**311**
RBC, 10^6/□L	7.6	7.6	7.9	8.1	7.0*	7.0	7.2	7.4	6.7	6.3**
MCV, fl	61	61	61	60	66**	59	60	59	61	67**
MCH, pg	19	19	19	19**	21**	19	19	19	19	21**
Reticulocyte, %o	22	27	22	24	65**	26	24	25	43**	94**
Heinz body, %o	1	2	5**	6**	151**	3	2	4	48**	298**

** p<0.01 by Mann, Whitney and Wilcoxon's U test.

Rats at 311 mg/m^3 showed slightly reduced plasma protein concentrations and T3 and T4 levels, accompanied by increased thyroxine binding capacity, suggesting a slight fall in thyroid function that was greater in males than in females. There was also a reduced trend in plasma urea and creatinine levels. The changes at 6.6 mg/m^3 were similar to those of solvent control group, and hence dismissed.

The liver N-demethylase activity was slightly increased at 311 mg/m^3 (M: 16 *vs* 10; F: 16 *vs* 10 nmol/g.min, both $p < 0.01$), suggesting induction of the microsomal mixed function oxidases of the liver. There were no abnormal findings in urinalysis.

Changes in Clinical Chemistry

Dose (mg/m^3)	male					female				
	Air	**Solv**	**6.6**	**48**	**311**	**Air**	**Solv**	**6.6**	**48**	**311**
Protein, g/L	59	56	58	56**	55**	57	56	56	55*	53**
Urea, mmol/L	7.8	7.0**	7.2**	6.6**	6.0**	7.3	8.3	6.9	6.4*	6.4*
Creatinine, □mol/L	65	58**	56**	50**	51**	80	78	62**	66	66
T3, nmol/L	1.0	0.9	0.9	0.9	0.8**	0.9	0.9	1.0	1.0	0.9
T4, nmol/L	63	65	62	64	53**	54	70**	59	55	41*
TBK	0.64	0.68	0.71	0.79**	0.84**	0.56	0.61	0.61	0.66**	0.67**

* p<0.05; ** p<0.01 by Mann, Whitney and Wilcoxon's U test.

Spleen weights were significantly increased in females at 48 mg/m^3 (20% higher) and both sexes at 311 mg/m^3 (33-38% higher). Necropsy revealed dark and swollen spleens with increased congestion at 311 mg/m^3.

The NOEL was 6.6 mg/m^3 based on changes in haematology (increased Heinz body and reticulocytes) and clinical chemistry (reduced protein, urea and creatinine levels, an increased TBK level), as well as increased spleen weights at higher concentrations.

Pauluhn J (1986b). Diuron: Study for subacute inhalation toxicity to the rat (aerosol exposure for 4 and 8 weeks). Lab: Bayer AG, Institute for Toxicology, Wuppertal-Elbertfel. Report No: 14603. GPL: yes. Guideline: OECD 412.

Methods
Wistar rats (5/sex/dose) were exposed head/nose only to 0 (solvent), 4.1, 37 or 268 mg/m^3 of diuron (Batch: 232114123, Purity: 98.4%) aerosol for 6 h per day, 5 days per week for 4 or 8 weeks. The rats (Gor: WISW, SPF-Cpb) were 8-10 weeks, 160 – 200 g and obtained from Breeder Winkel-Mann, Borchen, Kreis Paderborn, and feed with Altromin 1324 Diet. The solvent lutrol/ethanol aerosol, up to a nominal 20 mL/m^3, was tolerated by rats without undesirable effects. The aerosol concentrations of diuron and particle size (MMAD: approximate 2.2-2.4 □m with mean 88% ≤ 5 □m) were determined by sample analysis.

Body weights were determined weekly. Blood samples were collected by heart puncture at necropsy for examinations on haematology (see Appendix V, including Heinz bodies, Met-Hb and Sulpha-Hb), clinical chemistry (albumin, bilirubin, cholesterol, total protein, AP, ALT, AST, GLDH, TBK, T3 & T4, Na, K, Ca, Cl), and urine samples for urinalyses (blood, protein, glucose, pH, sediment, ketone, bilirubin, urobilinogen). Enzyme activities (Cytochrome P-450, N-demethylase and O-demethylase) in the liver were measured. Selected organs (adrenals, heart, kidneys, liver, lungs, spleen, thyroid, testes and ovaries) were weighed, all organs/tissues were grossly checked, and selected organs/tissues (femur and sternum, lung hilus lymph nodes, heart, testicles/ovaries, head, liver, lungs, spleen, adrenals, kidneys, trachea with larynx, thyroids with parathyroids, uterus, epididymis, urinary bladder and ureter) were histopathologically examined.

Results
All rats survived. Rats at 268 mg/m^3 showed transient lassitude and ungroomed coat post exposure. Lower body weight gain was seen in females at 37 mg/m^3 but not at the higher dose, which might not be related to treatment.

Heinz body formation, reticulocytosis and a slight reduction in RBC were observed at 37 (females) and 268 mg/m^3. The slightly increased MCV at 268 mg/m^3 indicated reduced stability and hence shortened erythrocyte life, and other alterations were attributed to secondary enhanced erythropoiesis. The findings are consistent with compensated slight haemolytic anaemia.

Changes in haematology (n = 5/sex)

Concentration (mg/m^3)	male				female			
	0	**4.1**	**37**	**268**	**0**	**4.1**	**37**	**268**
4 weeks								

RBC, 10^6/□L	7.8	7.6	7.2**	6.7**	7.0	6.6	6.3*	5.6**
Hb, g/L	152	145	142*	137**	134	130	125	119**
Hct	0.47	0.44	0.43**	0.43**	0.41	0.39	0.39	0.36*
MCV, fl	60	58	59	64	59	59	62	65*
MCH, pg	19.4	19.2	19.7	20.6	19.3	19.6	19.7	21.2**
Reticulocyte, %o	9	9	8	42**	20	19	25	64**
Heinz body, %o	0	0	5**	13**	1	0	1	8**
8 weeks								
RBC, 10^6/□L	8.8	8.5	8.4	7.4**	7.7	7.8	6.6**	6.2**
Hb, g/L	157	152	153	144**	141	142	130	127*
Hct	0.46	0.44	0.45	0.43	0.42	0.42	0.39	0.38
MCV, fl	53	52	53	57**	55	54	59*	61**
MCH, pg	18.8	17.8	18.2	19.4**	18.4	18.2	19.8*	20.4**
Reticulocyte, %o	15	21	9*	34**	13	11	42*	71**
Heinz body, %o	0	0	0	3	0	1	5*	17**

*p<0.01; ** p<0.01 by Mann, Whitney and Wilcoxon's U test.

The thyroxine level (T4) at 268 mg/m^3 was reduced, in particular significant in males (4 weeks: 47 *vs* 77; 8 weeks: 36 *vs* 52), indicating reduced thyroid activity. A significant rise of the O-demethylase activity in the liver after dosing with 268 mg/m^3 for 8 weeks (M: 16.8 *vs* 9.4; F: 15.0 *vs* 8.9 nmol/g.min, both $p < 0.01$) suggested enzyme induction. Histology did not however detect any pathological changes in thyroid or liver. There were no abnormal findings by urinalyses.

At necropsy, increased incidence of dilated hearts was seen in all treated groups without a dose-relationship. Dark and swollen spleens were observed at 37 mg/m^3 and above, correlated to increased spleen weight at 268 mg/m^3, and an increase in iron accumulation in the spleen at 37 (females) and 268 mg/m^3.

Spleen alterations (n=5/sex)

Concentration (mg/m^3)	**male**				**female**			
	0	**4.1**	**37**	**268**	**0**	**4.1**	**37**	**268**
Dark, swollen spleen (%), 4&8weeks			40	100			100	100
Weight (g), 4 week	514	525	462	778**	384	400	401	624**
8 week	446	490	433	587**	379	378	436	619**
Haemosiderin accumulation, 4 week				1+, 1++	4+, 1++	4+, 1++	1+, 4++	1+, 4++
8 week	2+	1+, 1++	3+	5++	5++	3+, 2++	1+, 2++, 2+++	4++, 1+++

+ mild; ++ moderate; +++ marked.

As shown in the table below, changes in urothelium of renal pelvis, urinary bladder and ureter, i.e. urothelial hyperplasia, were not dose-related, and could not simply attributed to the treatment.

Urothelial hyperplasia (n=5/sex)

Dose (mg/m^3)		**male**				**female**			
		0	**4.1**	**37**	**268**	**0**	**4.1**	**37**	**268**
4 week	Renal pelvis	1+	1+, 1++	3+,1+++	2+	2+	1+	1+	
	Urinary bladder		2+	3+, 1+	1+	2+	3+, 1++	3+, 1++	3++
	Ureter		3+, 1++	1+, 1++	3+, 1++	3+	2+	2+	1++
8 week	Renal pelvis	1+	5+	2+, 1++		2+		1+	1+

	Urinary bladder	2+,1++	2+	1+, 1++		2++	2+	3+, 1++	4+
	Ureter	1+	1++	2+	2+	2+	2+	1+, 1++	3+

Normal: up to 4 layer cells; +: 4-6 layer cells; ++: 6-9 layer cells with preponderance; +++: over 9 layer cells with barely visible or missing surface cell layer.

The lung findings, including peribronchial round cell infiltration, margin distension and/or focal vascular calcification were found in all groups, and might not be not related to the treatment.

The NOEL was 4.1 mg/m^3, based on haematological (reduction in RBC, haemoglobin and haematocrit, increase in Heinz body and reticulocyte) and related pathological changes in the spleen (haemosiderin accumulation) at higher concentrations.

5. SUBCHRONIC STUDIES

5.1 Oral Administration

Rats

Wandrag S (1996a). Subchronic oral toxicity-rodent: 90-day study with samachem diuron in rats. Lab: Biocon Research (Pty) Ltd, Pretoria, South Africa. Sponsor: Sanachem (Pty) Ltd, Durban, South Africa. Study No: 48716. Study Duration: June – September 1994. GLP/QA: yes. Guideline: OECD 408.

Methods
Sprague Dawley rats (12/sex/dose) received diuron technical at 0, 75, 250 or 500 mg/kg by gavage over 5 days/week for 13 weeks. The test substance (Batch No: 530.4.94, Purity: 98.5%, from Sanachem Pty Ltd, Durban, South Africa) was mixed with cottonseed oil within 4 hours prior to dosing. Rats were received from Roodeplaat Research Laboratoies Pty Ltd, and were approximately 8 weeks old and 85-115 g for males and 80-104 for females at start of the study.

Clinical observation was made daily, and body weight measured weekly. Blood samples were collected for laboratory examinations including haematology (excluding clotting parameters and blood smear) and clinical biochemistry (Appendix VI, excluding ALP, cholesterol, globulin, LDH, CPK, triglycerides) at the last day before euthanasia. No urinalyses were performed. On study termination, all surviving rats were sacrificed, selected organs (liver, kidneys, adrenals and testes) were weighed, and tissues (adrenals, aorta, bone marrow, brain, heart kidneys, large and small intestine, liver, lungs, lymph nodes, nerve, oesophagus, pancreas, spleen, sternum, stomach, testes, thymus, thyroid, trachea, urinary bladder) were examined histopathologically.

Results
Two accidental deaths each occurred in the male control group and the female 500 mg/kg group. There were no other mortalities and no treatment-related clinical signs observed. Lower body weight gain was seen in males at 250 (8% lower) and 500 mg/kg bw/day (20% lower).

Laboratory examinations revealed treatment-related changes in haematology, including reduction in RBC counts, haemoglobin and haematocrit, and increase in MCV, and suggesting haemolytic anaemia.

Changes in haematology (n = 12/sex)

	male				**female**			
mg/kg bw/day	0	75	250	500	0	75	250	500
RBC, 10^6/□L	8.5±0.6	7.5±0.4	7.0±0.3	6.5±0.5	8.5±0.4	6.6±0.4	6.3±0.3	6.1±0.4
Hb, g/L	16.7±0.6	15.3±0.6	14.9±0.5	14.9±1.2	17.0±0.6	14.7±0.5	14.9±0.6	14.8±0.9
Hct, %	50.5±2.1	46.4±2.2	45.9±1.7	44.6±3.3	56.1±2.3	49.2±2.6	50.8±2.4	50.7±3.5
MCV, fl	59.4±3.4	61.6±1.5	65.2±1.3	68.7±1.5	66.4±2.1	74.5±1.8	80.4±1.8	83.2±2.2

Statistics analysis described by the study author was not clear.

A dose-related increase in total serum bilirubin and serum urea was observed was significant at some high dose groups.

Changes in clinical chemistry (n = 12/sex)

	male				**female**			
mg/kg bw/day	0	75	250	500	0	75	250	500
Total bilirubin, □mol/l	2.3±0.3	2.9±0.8	4.3±0.5	6.0±0.9 *	2.6±0.4	4.0±0.9	5.2±0.8 *	6.3±0.6 *
Urea, mmol/l	6.8±0.9	7.8±1.2	8.4±0.8	11.1±1.6*	6.7±0.7	7.4±1.0	8.7±1.6 *	9.8±1.0 *

* $P \leq 0.05$ by Student's t-test.

The post mortem examinations revealed splenomegaly (enlarged spleen) in 2 males and 1 female at 75 mg/kg bw/day, 6 males at 250 mg/kg bw/day, and 11 males and 2 females at 500 mg/kg bw/day, which were consonant with haematological findings. No treatment-related changes were found in organ weights and histopathology.

A NOEL was not established in this study due to changes in haematology (reduction in RBC, haemoglobin and haematocrit, an increase in MCV) and clinical chemistry (increased blood bilirubin and urea), as well as enlarged spleen at all dose levels. The LOEL was 75 mg/kg bw/day.

Beres E (1986). 90 days feeding study of diuron technical active ingredient on Wistar rat. NEVIKI Research Institute for Heavy Chemical Industries. Veszprem.

Methods
Wistar rats (20-40/sex/dose) received a daily dose of diuron at 0, 55, 168 (females), 225 (males), 335 (females) or 450 mg/kg bw/day (males) in the diet for 90 days, followed by a 30-day post-observation period for the groups at 0 335 and 450 mg/kg bw/day. Clinical signs were observed daily, and body weights measured weekly. Neurotoxicological tests, including motor coordination and orientation hypermotility, were performed at 45 days and 90 days post-dosing. Blood samples were taken by heart puncture under ether narcosis at termination, for haematology (RBC, WBC, haematocrit, haemoglobin, platelet) and clinical chemistry examinations (bilirubin, urea nitrogen, LDH, AST, ALT, cholinesterase, methemoglobin). Selected organs (no details) were weighed.

Results
There were no deaths. The extremities of the treated rats, including the ears, nose, perioral region and tail, became pale after 3-4 week treatment, but appeared reversible at the end of the post-observation period. Body weight was slightly lower (5-9%) at 55 mg/kg bw/day and significantly lower (7-21%) at 225 mg/kg bw/day and above. Reduced RBC count, haemoglobin and haematocrit, and increased bilirubin and methemoglobin, increased spleen and pituitary weights were reported but no data was presented. Results from neurotoxicological tests were not presented.

Since there was insufficient information provided for an independent assessment of the data, this study is not considered suitable for regulatory purposes.

5.2 Dermal Application

Wandrag S (1996b). Subchronic dermal toxicity-rodent: 90-day study with Sanachem diuron technical in rats. Lab: Biocom Research (Pty) Ltd, Pretoria, South Africa. Sponsor: Sanachem (Pty) Ltd, Durban, South Africa. Study Duration: July-October 1994. GLP/QA: yes. Guideline: OECD 411.

Methods
Sprague Dawley rats (12/sex/dose) received dermal applications of diuron Technical at 0, 250, 500 or 1000 mg/kg on clipped skin of the dorsal area of the trunk over 5 days/week for 13 weeks. The test substance (Batch No: 530.4.94, Purity: 98.5%, from Sanachem (Pty) Ltd, Durban, South Africa) was mixed with cottonseed oil to a concentration of 254 $\pm$ 4 mg/mL (by analysis) within 4 h prior to dosing. Rats were supplied by Roodeplaat Research Laboratories Pty Ltd, and were approximately 10 weeks old, and 202-262 g for males and 216-298 g for females at the start of the study.

Clinical observations were made daily, and body weight measured weekly. Ophthalmoscopic examinations were made before treatment and at termination. Blood samples were collected before euthanasia for laboratory examinations including haematology (excluding ALP, cholesterol, globulin, LDH, CPK, triglycerides) and clinical biochemistry (excluding clotting parameters and blood smear). No urinalyses were performed. On Day 91/92, all rats were sacrificed and grossly examined, selected organs (liver, kidneys, adrenals and testes) were weighed, and organs/tissues (adrenals, aorta, bone marrow, brain, heart kidneys, large and small intestine, liver, lungs, lymph nodes, nerve, oesophagus, pancreas, skin, spleen, sternum, stomach, testes, thymus, thyroid, trachea, urinary bladder) were examined histopathologically.

Results

One control male was killed by humane euthanasia due to otitis media. Three deaths each occurred at 500 (1 male) and 1000 mg/kg bw/day (1 male and 1 female) during Days 53-60 without showing any symptoms prior to death. The cause of deaths was not established. Histopathology revealed severe cystitis in urinary bladder and/or mild atrophy testicular in the treated males, and severe necrosis liver in the female, the later might be related to the death. There were no treatment-related clinical signs observed in survivors.

Mean body weight gain was comparable between groups. There were no ophthalmoscopic findings. Laboratory tests revealed changes in haematology parameters, reduced RBC count, haemoglobin and haematocrit, and an increase in MCV and MCH.

Changes in haematology (n = 12/sex)

	male				**female**			
mg/kg bw/day	0	250	500	1000	0	250	500	1000
RBC, $x10^6$	8.6±0.3	7.5±0.5	7.4±0.2	7.2±0.4	8.5±0.4	6.2±0.5	6.0±0.4	6.1±0.5
Hb, g/L	16.2±0.5	15.0±0.8	15.0±0.5	14.6±0.7	16.1±0.8	14.0±1.2	13.5±0.8	13.6±1.0
Hct, %	51.2±2.1	47.3±3.4	48.0±1.1	46.8±2.3	49.8±3.3	44.1±4.6	43.1±2.9	43.7±3.6
MCV, fl	59.8±1.5	63.3±1.3	64.4±1.4	65.3±1.5	62.2±0.9	70.6±2.3	71.9±1.8	71.5±1.6
MCH, pg	19.0±0.3	20.1±0.6	20.1±0.5	20.4+0.4	20.2+0.3	22.4±1.0	22.6±0.6	22.3±0.6

Results expressed as the mean ± SD;
Statistics analysis described by the study author was not clear.

Increased total blood bilirubin was observed in all treated groups, without a dose-relationship. Blood urea nitrogen was lower in some treated groups.

Changes in clinical chemistry (n = 12/sex)

	male				**female**			
mg/kg bw/day	0	250	500	1000	0	250	500	1000
Total bilirubin, □mol/l	2.5±0.3	3.1±0.4	2.9±0.5	3.0±0.7	2.1±0.4	3.4±0.7 *	2.9±0.8 *	3.8±1.0 *
Urea, mmol/l	7.6±1.2	7.0±1.3	6.3±1.1 *	6.4±1.4 *	8.0±1.0	7.9±0.7	7.4±0.6	7.4±0.8

Results expressed as the mean ± SD;
* P ≤ 0.05 by Student's t-test.

Urolith was found in some male rats of all groups. No treatment-induced organ weight changes or histopathologic alternations were observed in any tissues including the bone marrow.

A NOEL was not established in this study due to reduced RBC, haemoglobin and heamatocrit, and elevated MCV and MCH at all dose levels tested. The LOEL was 250 mg/kg bw/day.

6. CHRONIC STUDIES

6.1 Oral Administration

Mice

Eiben R, Kaliner G, Karbe E & Suberg H (1990). Diuron: Chronic toxicity and carcinogenicity with NMRI mice (Administration in diet for 24 months). Lab: Bayer AG, Institute for Toxicology,

Wuppertal, Friedrich-Ebert-Strasse 271-233, West Germany. Report-no: 51492-51494. Guidelines: USEPA 83-1, 83-2. Study Duration: October 1981 – October 1983. GLP/QA: yes.
&
Hardesty PT & Pelt DS van (1994). Supplementary data supporting the diuron 2-year feeding study in NMRI mice. Lab: El Du Pont de Nemours and Company , Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, PO Box 50, Newark, Delaware 19714. Report-no: 51519. Guidelines: USEPA 83-1, 83-2. GLP/QA: yes.
&
Malek DE (1997). Cancer hazard characterization of diuron. El du Pont de Nemours and Company, Dupont Agricultural Products, Barley Mill Plaza, Wilmington, Delaware 19880-0038, USA. Report no: 51520.

Methods
NMRI mice (60/sex/dose) received diuron at 0, 25, 250 or 2500 ppm (0/0, 5.4/7.5, 50.8/77.5 or 640/876 mg/kg bw/day for M/F by diet analysis) in the daily diet for 12 or 24 months. Satellite groups (10/sex/dose) were sacrificed after 12 months. Mice were supplied by SPF-bred by Winkelmann, Borchen, and were 7 weeks old, 19-33 g for males and 19-27 g for females at the beginning of dosing. The dose levels were determined by a ranging finding study. The mixture of diet (Altromin 1321 meal, ad libitum) with the test substance (Batch No: 232114080, Purity: 98.7%) was prepared weekly and analysed at 3-month intervals.

Clinical observations were made daily, detailed individual inspections, food consumption and body weight weekly (except biweekly for body weight during Weeks 28 to 37). Blood samples (taken under ether anaesthesia except those for testing plasma glucose) were collected from 10 mice/sex/dose after 6, 12, 18 and 24 months of dosing, for haematology (Appendix V including RBC morphology and excluding clotting parameters) and clinical biochemistry (ALP, ALT, AST, LDH, cholesterol, creatinine, globulin, urea, glucose, cholesterol, bilirubin, total protein). No urinalyses were performed in this study. Selected organs (adrenals, heart, liver, lung, spleen, kidneys, testicles,) from all interim and main groups were weighed. At interim sacrifice, organs/tissues at 0 and 2500 ppm, and livers, kidneys, lungs, spleen (except 25 ppm) and all macroscopically changed organs from all groups were microscopically examined. All organs/tissues (Appendix VI, excluding oviduct and mesenteric lymph node) from main groups were examined microscopically. In addition, uteri were morphometrically measured, and spleens were densitometrically measured.

Results
There were no treatment-related increases in mortality, clinical signs and changes in food consumption. Body weights were significantly lower (up to 8% and 11% for males and females) at 2500 ppm.

Erythrocyte parameters show no consistent and significant changes during the 2-year study.

Mice at 2500 ppm showed pathological changes in the liver such as hepatopathy, hepatocellular enlargement, increased rates of mitosis and cell necrosis, indicating liver injury. The centrilobular hepatopathy which developed in males of this group was characterised by enlarged liver cells, the cytoplasm of which was finely granulated and eosinophilic in appearance or contained a number of small nuclei which probably derived from Kupffer cells and might signal the imminent death of the affected liver cells. The enlarged liver cells or surrounding phagocytes contained yellow pigment. The number of hepatic lobules affected by this centrilobular hepatopathy varied enormously from section to section. The mitosis rate of liver cells in males of this group was high, and single cell necrosis and the accumulation of Kupffer cells were observed slightly more frequently than in other groups. In addition to a higher incidence of mitoses and cell necroses also in females at 2500 ppm, a trend to an increased incidence of liver cell enlargement was observed in all treated females groups in a dose-related manner.

Increased number of mice at 2500 ppm developed grade 2-3 of haemosiderin accumulation in hepatocytes and phagocytes. Haemosiderin deposition was also observed in the spleen and kidneys (females) at 2500 ppm, at the terminal as well as interim sacrifice.

Histopathological findings in the liver, spleen and kidneys (n = 35-48/sex)

Dose (ppm)	male				female			
	0	**25**	**250**	**2500**	**0**	**25**	**250**	**2500**
Liver								
Enlarged degenerative cells, no of mice	0	0	0	0	0	1	3	10**
Hepatopathy, no of mice	1	0	0	15**	0	0	0	0
Increased mitoses, no of mice	1	2	0	8**	0	3	0	4*
Single cell necroses, no of mice	3	2	5	7*	12	7	10	19**
Accumulation of Kupffer cells, no of mice	6	6	8	11*	9	9	9	9
Haemosiderin in hepatocytes (0-1 degree), %	97	-	93	82	-	-	-	-
(2-3 degree), %	3	-	7	18	-	-	-	-
Haemosiderin in phagocytes (0-1 degree), %	86	-	83	73	-	-	-	-
(2-3 degree), %	14	-	17	27	-	-	-	-
Spleen								
Golden-brown pigment, no of mice	1	0	1	14**	6	2	6	19**
Haemosiderin-deposition, %	70	-	81	84	87	-	83	90
Haemosiderin-deposition (0 degree), %	30	-	19	16	13	-	17	10
(1 degree), %	51	-	51	38	58	-	48	34
(2 degree), %	16	-	26	22	16	-	26	39
(3 degree), %	3	-	2	13	7	-	9	12
(4 degree), %	0	-	2	11	7	-	0	5
Kidneys								
Golden-brown pigment, no of mice	0	0	0	0	0	1	1	5**
Haemosiderin-deposition, %	33	44	70	58	73	-	67	74
Haemosiderin-deposition (0-1 degree), %	89	88	83	84	60	-	56	46
(2-4 degree), %	11	13	17	16	40	-	44	54

***p<0.05, **p<0.01 by *Mann-Whitney U-test*. - : Not examined.**

Females, but not males, at 2500 ppm exhibited an increase in epithelial hyperplasias of the bladder, associated with extension of the lamina propria mucosae in some cases, and often accompanied by mucosal oedema. Epithelial hyperplasia of the bladder was already apparent after 12-month of treatment.

Microscopical findings in the urinary bladder at 24-month

Dose (ppm)	male				female			
	0	**25**	**250**	**2500**	**0**	**25**	**250**	**2500**
n	44	47	47	46	46	36	45	44
Epithelial hyperplasia, no of mice	14	12	13	12	5	5	3	23**
Oedema	0	0	0	0	0	0	0	17**
Mucosa thickened	0	0	0	0	0	0	0	5**

****p<0.01 by *Mann-Whitney U-test*.**

At 2500 ppm, there was a slight increase in the number of females which displayed increased diameter of the uterine horn, in the absence of hyperplasias. A significant increase in the incidence of ovary luteoma was also observed at 2500 ppm. However, the incidence of combined sex cord-stromal tumours (including granulosa cell tumours, luteomas, thecomas, Sertoli cell tumours of the ovary, Leydig cell tumours, androblastoma, arrhenoblastoma and lipid cell tumours) was comparable across the groups, and within the range of historical control.

Incidences of neoplasms

	female

Dose (ppm)	**0**	**25**	**250**	**2500**	**Range of historical control**
No of mice examined [1]	50 / 39	47 / 32	49 / 44	50 / 39	
Ovary, unilateral luteoma, no (%)	3 (6)	0	2 (4)	7 (14)**	
Combined sex cord-stromal tumours [2]	11 (22)	7 (15)	15 (31)	14 (28)	0-35.5% (reported)
Mammary, adenocarcinoma, no (%)	2 (4)	1 (2)	1 (2)	6 (12)*	Max. 13% [3]

*** p<0.05, **p<0.01 by *Cochran-Armitage Trend Test*.**

[1] the number of mice for gross examination / the number of mice for microscopic examination.

[2] The US National Toxicology Program recommended a combination of the total incidence of the sex cord-stromal tumours for statistical assessment of tumour data. The historical range derived from literature (Rehm et al, 1984; Bomhard & Mohr, 1989; Lohrke et al, 1984; Rehm et al, 1985).

[3] The sponsor provided published historical data from the Bayer Ag laboratory with a historical control up to 13% (Bomhard & Mohr 1989; Bomhard 1993). A published historical control range in Han:NMRI female mice: 9-14% in ad libitum fed mice and 2-9% in food restricted fed mice (Rehm et al, 1985) is also provided.

Mammary gland tumours at 2500 ppm were significantly higher than the concurrent control, but were within the range of historical control in Han:NMRI mice from the same test facility (up to 13%) and from other laboratories (9-14%).

There were no significant differences in the number of animals with neoplasms, the total number of neoplasms, number of malignant or benign neoplasms between treated groups and control.

The NOEL for general toxicity was 250 ppm (51/78 mg/kg bw/day for M/F) in this study based on hepatopathy, mitoses, cell degeneration and necroses, and Kupffer cell accumulation in the liver, and haemosiderin deposition in the liver, spleen and kidneys at the higher dose. The NOEL was 250 ppm for bladder epithelium hyperplasia, and was 2500 ppm for carcinogenicity, the highest dose.

Rats

Schmidt WM (1985). Diuron: Study for chronic toxicity and carcinogenicity with Wistar rats (Administration in diet for up to two years). Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Report No: 51470. Du Pont Report No: D/Tox 17. Study Duration: September 1981 – September 1983. GLP/QA: Yes. Guideline: US EPA 83-1.

&

Rossberg WM (1995). Volume 1 of Supplementary data supporting the diuron 2-year feeding study in rats. Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Report No: 51481.

&

Malek DE (1997). Volume 2 of Supplementary data supporting the diuron 2-year feeding study in rats. Lab: El du Pont de Nemours and Company, DuPont Agricultural Products, Barley Mill Plaza, Wilmington DE 19880-0038, USA. Report No: 51484.

&

Rossberg WM & Wirnitzer U (1994). Study for chronic toxicity and cancerogenicity with Wistar rats (Administration in diet for up to two years, Addendum to Bayer-report no. 13962 dated October 10, 1985). Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Report No: 6548.

&

Rossberg WM & Wirnitzer U (1995). Study for chronic toxicity and cancerogenicity with Wistar rats (Addendum 1). Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Report No: 51482.

&

Rossberg WM & Eiben R (1997). Study for chronic toxicity and cancerogenity with Wistar rats (Administration in diet for up to two years – Second amendment). Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Report No: 51483.

&

Heimann KG (2001). Diuron: assessment of reversibility of urinary bladder effects after chronic oral application to rats. Lab: Bayer AG, Business Group Crop Protection, Development / Registration, D-51368 Leverkusen, Germany.

Methods
Wistar rats (60/sex/dose) received diuron at 0, 25, 250 or 2500 ppm (0/0, 1.0/1.7, 10/17, 111/203 mg/kg bw/day for M/F) in the daily diet for up to 24 months (104 weeks). Ten rats per group (satellite) were sacrificed after 12 months. Rats (SPF Cpb) were 6-7 weeks old, and mean weight 85 g for males and 84 g for females at the beginning of dosing. Fresh powdered feed (Altromin 1321, made by Altromin GmbH, Lege, ad libitum) mixtures with the test substance (Batch No: 232114080, Purity: 98.7%) were prepared fresh weekly, and the stability, homogeneity and concentration of the test substance in the diet were analysed.

Clinical observations were made daily, detailed individual inspections and food consumption weekly, and body weight weekly up to week 27 then biweekly thereafter. Blood (under ether anaesthesia except those for testing plasma glucose) and urine samples were collected from 10 rats/sex/dose after 6, 12, 18 and 24 months of dosing, for haematology (Appendix V), clinical biochemistry (ALP, ALT, AST, creatinine, urea, glucose, cholesterol, bilirubin, total protein) and urinalyses (glucose, blood, protein, bilirubin, ketone bodies, pH, urobilinogen sediment, protein). Rats were sacrificed by exsanguination under anaesthesia with diethyl ether after 12- or 24- month dosing and grossly examined, and selected organs (adrenals, heart, liver, lung, spleen, kidneys, testicles) were weighed. From rats of the main groups sacrificed after 24 months and from those which died spontaneously or were moribund and sacrificed during the study, organs/tissues (Appendix VI, including larynx and vertebral column, and excluding gall bladder, skin, vagina) were examined histopathologically, and tissue sections of spleen and bone marrow were examined morphometrically. Histopathological examination were also performed on selected tissues (lungs, liver, spleen, bone marrow, kidneys, renal pelvis, urinary bladder, sternum and femur) of all dose groups, and other tissues from 0 and 2500 ppm groups at 12- month interim sacrifice. It is noted that mammary glands were preserved but not routinely evaluated.

Results
The mortality rate was generally low across groups (up to 22% by the end of study), and not affected by treatment. Neoplasias (malignant carcinoma in the urinary bladder in males and uteri neoplasias in females, see the below for details) could be the reason for premature deaths/sacrifices in most cases.

From approximately week 10 onwards, body weight was lower in males at 250 ppm (up to 5% lower) and in both sexes at 2500 ppm (up to 20% lower). Food consumption was comparable among the groups. It is noted that compared to the intake of the test substance by females which were consistent with that in another study from the same laboratory (Schmidt & Karbe, 1986), male groups received considerable higher doses in relation to the body weights (0/0, 1.0/1.7, 10/17 and 111/203 mg/kg bw/day for M/F at 0, 25, 250 and 2500 ppm respectively). It is shown from the original data that males and females had a ratio of body weights at approximate 3:2, but consumed similar amount of food mixed with the test substance.

There were dose-related changes in erythrocyte parameters in all treated female groups, and in males at 2500 ppm throughout the study and at 250 ppm at various testing times, suggesting haemolytic anaemia. Leucocyte counts were increased at 250 (males) and 2500 ppm, possibly as a result of erythrocyte damage. These effects were accompanied by increased incidences of erythrocyte precursors (reticulocytes) and pathological erythrocytes (anulocytosis, anisocytosis, polychromasia, Howell-Jolly bodies and Heinz bodies) in these groups. Alternations with values outside or at the limit of the reference range were only observed in males at 2500 ppm, and in all treated female groups.

Changes in haematology after 24-month treatment (n = 10/sex)

	male				**female**			
Dose (ppm)	**0**	**25**	**250**	**2500**	**0**	**25**	**250**	**2500**
RBC, 10^6/□L	8.24	8.14	7.90	6.82**	7.69	6.86*	6.40**	5.95**
Haemoglobin, g/L	15.5	15.8	15.1	14.3*	15.5	14.4	14.1**	13.8**
MCV, fl	57	58	59	65**	59	63*	65**	67**
Reticulocyte, %o	17	22	21	72**	17	23*	30**	62**

Haematocrit,	0.47	0.47	0.46	0.44	0.45	0.43	0.42**	0.40**
MCH, pg	18.8	19.5	19.1	20.9**	20.2	21.2*	22.1**	23.2**
Leucocyte, 10^3/□L	6.7	6.1	8.6**	11.3**	7.3	7.3	6.1	13.4**

***p<0.05, **p<0.01 by *Mann-Whitney U-test*.**

The plasma bilirubin concentration was consistently higher in male and females at 2500 ppm. The blood urea concentration was also increased at 2500 ppm, and sometimes at 250 ppm.

Changes in clinical chemistry after 24-month dosing (n = 10)

	male				**female**			
Dose (ppm)	**0**	**25**	**250**	**2500**	**0**	**25**	**250**	**2500**
Bilirubin, □L/L	2.8	2.6	3.0	4.2**	3.3	3.2	2.6*	4.6**
Urea, mmol/L	5.68	5.88	6.44	7.68**	6.86	7.07	7.65*	8.49**

***p<0.05, **p<0.01 by *Mann-Whitney U-test*.**

The reddish discoloration of the urine was noted with a higher incidence in males at 2500 ppm (28 *vs* 1 in control), particularly in the second year. Urinalysis revealed a higher blood/erythrocyte content in males and females at 2500 ppm.

Liver and spleen weights were significantly increased in males at 250 and 2500 ppm, and in all treated female groups after 24-month dosing, and to a lesser extent after 12-month dosing. At terminal necropsy, swelling/enlargement and black coloration of the spleen were found in females of these groups. Morphometric examination revealed increased haemosiderin accumulation in the spleen of males and females at 250 and 2500 ppm accompanied by enhanced medullary erythropoiesis. Haemosiderin accumulation was also seen in these male groups at interim sacrifice. Fibrosis of the spleen was observed at 250 and 2500 ppm, which was connected with the harmful effect on the erythrocytes.

Organ weights

		male				**female**			
Dose (ppm)		**0**	**25**	**250**	**2500**	**0**	**25**	**250**	**2500**
12 months	Body, g	384	393	373	355	211	220	210	191**
	Liver, g	13.4	14.1	13.0	13.4	7.8	8.6	7.7	7.8
	% body	3.5	3.6	3.5	3.8	3.7	4.0	3.6	4.2**
	Spleen, g	0.60	0.72**	0.71	1.33**	0.41	0.46	0.63**	11.7**
	% body	0.16	0.18**	0.19**	0.38**	0.19	0.21	0.30**	0.62**
24 months	Body, g	421	418	401*	354**	249	256	257	220**
	Liver, g	15.0	14.9	15.4	14.5	8.3	9.9**	9.2**	9.8**
	% body	3.6	3.6	3.8**	4.1**	3.3	3.9**	3.6**	4.5**
	Spleen, g	0.79	0.84	0.88**	1.51**	0.49	0.72**	0.80**	1.26**
	% body	0.18	0.20	0.22**	0.43**	0.20	0.28**	0.31**	0.57**

***p<0.05, **p<0.01 by *Mann-Whitney U-test*.**

Morphometric evaluation of specimens of the femoral bone marrow showed increased medullary haematopoiesis (increases in surface areas of activated bone marrow or fall in surface areas of fat marrow) at 250 and 2500 ppm. The overall findings suggested non-progressive haemolytic (hyperchromic) anaemia. There were no indications of primary damage to blood-forming or blood regenerating tissue. In addition, correlating with the dark discolouration and higher liver weights, the histopathological examination detected slightly increased deposition of iron-containing pigments in the liver, kidneys and lungs, particularly at 2500 ppm.

Morphometric findings of spleen and bone marrow (24 months)

Dose (ppm)	male				female			
	0	**25**	**250**	**2500**	**0**	**25**	**250**	**2500**
Spleen, % fibrosis	0	0	6	37	0	0	0	34
haemosiderin particles, % surface area	6.8	7.6	14.0	12.9	9.2	12.7	17.7	18.0
Bone marrow, activated haematopoietic, % surface	56.8	57.4	61.0	81.4	50.4	51.3	71.1	80.3
fat marrow, % surface (12 month)	33.6	31.9	23.0	n.a.	29.2	29.7	11.3	5.4

n.a.: not evaluated.

Hardness and/or thickening of the urinary bladder wall appeared at 2500 ppm. An increased extent and higher incidence of hyperplasia of the urinary bladder epithelium was observed in females at 250 ppm after 24-month dosing, and in males and females at 2500 ppm after 12- and 24-month dosing. Similarly, an increased extent and a higher incidence of hyperplasia of the renal pelvis epithelium were also detected in males after 12-month dosing at 2500 ppm, and in both sexes after 24-month dosing at 250 and 2500 ppm.

Histopathological findings (24 months, n=48-50 unless specified)

Dose (ppm)		male				female			
		0	**25**	**250**	**2500**	**0**	**25**	**250**	**2500**
Spleen	Activated		2	1	2	4	9	1	2
	Hyperaemia			1	15		1		2
	Congestion				1				
	Fibrosis			3	18				17
Bone marrow	Activated		5	7	24	5	12	22	42
	Increased fat marrow	5	10	2	1	2	5		
	haemorrhage	8	2	2	2	1	1	1	1
Kidneys	Round cell infiltration	3	12	9	31	1	3	2	7
Renal pelvis, 12 M	Urothelial hyperplasia, 1^o	5	3	6	5	1	2	5	4
	Urothelial hyperplasia, 2^o		1		5		1		
	Urothelial hyperplasia, 3^o						1		
24 M	Urothelial hyperplasia, 1^o	31	30	18	3	20	22	12	5
	Urothelial hyperplasia, 2^o	5	7	25	23	3	3	30	33
	Urothelial hyperplasia, 3^o	1	0	2	17			4	4
Bladder, 12 M	Urothelial hyperplasia, 1^o	3	5	4			3	5	5
	Urothelial hyperplasia, 2^o			1	2				1
	Urothelial hyperplasia, 3^o				5				2
	Papillary urothelial hyperplasia, 2^o	1							
	3^o				3				1
24M	Urothelial hyperplasia, 1^o	11	5	15	1	10	7	9	4
	Urothelial hyperplasia, 2^o	2	0	1	3	1		3	17
	Urothelial hyperplasia, 3^o				10			5	9
	Urothelial hyperplasia, 4^o				1				

1^o to 4^o: degree 1 to degree 4; 4-6 cell layers in normal stratification as 1^o; 6-9 cell layers and distinct preponderance of small cells as 2^o; > 9 cell layers with deep growth as 3^o; degree 3 with atypia as 4^o.

The total number of neoplasias and number of animals with neoplasias were higher at 2500 ppm, in particular males of this group, which is attributed to the higher incidence of malignant neoplasias. The commonest neoplasias in animals which died or were sacrificed during the study were carcinomas of the males' urinary bladders, and malignant neoplasias of the females' uteri. Only one neoplastic alteration (a benign ovary tumour at 2500 ppm) was found at interim sacrifice. The earliest time when a neoplasia was detected was 16 months for carcinoma of pituitary in a male at 250 ppm, and 12 months for adenocarcinoma of mammary in a female at 25 ppm.

Neoplasia findings (24-month treatment)

Dose (ppm)	male				female			
	0	**25**	**250**	**2500**	**0**	**25**	**250**	**2500**
Number of rats examined	50	50	50	49	48	50	50	50
Total neoplasias	21	16	19	57	31	31	31	39
Including: benign	18	12	11	18	23	20	19	19
malignant	3	4	8	39	8	11	12	29
Number animals with neoplasias	19	14	16	41	26	27	22	29
Number animals with multiple neoplasias	2	2	3	13	4	4	7	9
Number animals with only benign neoplasias	17	10	8	4	18	16	10	4
Number animals with only malignant neoplasias	2	2	6	25	6	10	6	19
Number animals with benign and malignant neoplasias	0	2	2	12	2	1	6	6

A higher incidence of malignant transitional carcinomas in the urinary bladder epithelium was observed in males and females at 2500 ppm, some with cornification or occasionally with differentiation from squamous epithelial carcinoma. No such tumours were found at 12-month interim sacrifice. Benign neoplasia of urinary bladder, papillomas were slightly higher in males at 2500 ppm. It was suggested that severe hyperplasia was a possible precursor of the neoplastic alteration.

Neoplasia findings in the main group (as %, n = 48-50/sex)

Dose (ppm)		male				female			
		0	**25**	**250**	**2500**	**0**	**25**	**250**	**2500**
Bladder	Transitional epithelial papilloma				6	2		4	4
	Transitional epithelial carcinoma	2		2	67				22
Kidneys	Transitional epithelial papilloma				2				
	Transitional epithelial carcinoma				4				
Uterus	Squamous cell carcinoma							2	2
	Endometrium sarcoma								4
	Adenocarcinoma					10	10	10	18

No statistics were presented.

It is notable that the distribution of neoplasias of the uterus showed an increase in the number of adenocarcinomas and endometrium sarcomas at 2500 ppm. However, a relatively high spontaneous incidence of adenocarcinomas in the uterus in historical control studies (2-20%, Kroetlinger, Institute of Toxicology, Bayer AG, 1985) was provided by the sponsor.

A higher incidence of adenocarcinoma in the mammary gland was found later in a 2-year mice study (Eiben et al, 1990), whereas mammary glands were not histopathologically examined in this rat study. In an attempt to fill in this data gap, histopathological examination on residual mammary tissue attached to the salivary glands from interim groups at 0 and 2500 ppm and from all main groups were conducted, and did not reveal any treatment-related abnormality (Rossberg & Eiben 1997; Malek 1997).

A NOEL for the general toxicity was not established due to haematological changes (decreased RBC, haemoglobin and haematocrit, and increased MCV, MCH and reticulocyte), increased liver and spleen weights, haemosiderin accumulation in the spleen and haematopoietic changes in the bone marrow in females at 25 ppm (these changes were not seen in males at 25 ppm, probably due to a lower intake of the test substance) and in both sexes at higher dose levels. The LOEL was 25 ppm (1.0/1.7 mg/kg bw/day for M/F). The NOEL for carcinogenicity was 25 ppm (1.0/1.7 mg/kg bw/day for M/F) based on transitional epithelial carcinoma in the urinary bladder and kidneys at 2500 ppm and urothelial hyperplasia as the potential precursor of neoplasia at 250 ppm and above.

Hodge H C & Downs W L (1964). Chronic feeding studies of diuron in rats. Department of Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA. Report No: 51486. MRID No: 17764. Study Duration: June 1960 – June 1962. Guidelines: no. GLP: no.

Methods
Rats (35/sex/dose) received diuron (as a wettable powder of 80% diuron) at 0, 25, 125, 250 or 2500 ppm in the daily diet for 24 months. The dose levels were determined by a pilot study. The dietary mixtures (Purina Fox Chow Meal) with the test substance (Code T 71113D, received from E I DuPont de Nemours and Company, Inc. Wilmington, Delaware) were prepared weekly.

The rats were observed for mortality, regularly for general clinical condition and appetite, after 1 and 4 months for food consumption, and weekly for body weight during the first 3 months and fortnightly thereafter. Blood samples were collected from 5/sex/dose group pre-test, monthly for the first 6 months, every 2 months for the next 6 months, and every 3 months during the second year, and from all rats at termination for haematological examinations (RBC counts and numbers of RBC characteristics, haemoglobin, total and differential WBC counts). Urine samples were collected at least 3 times a year for semi-quantitative determination of sugar and protein. At the time of autopsy, organ weights (liver, kidneys, lungs, brain, heart, spleen, testes) from all rats, gross- (all organs) and histo-pathology (brain, lungs, heart, liver, spleen, kidneys, adrenals, gonads, stomach, large and small intestines, urinary bladder, bone marrow, muscle and thyroid) from 10/sex/dose were examined. At termination, pooled samples of tissue (muscle, peri-renal fat, liver, kidney, spleen, blood), urine and faeces were collected for analyses of the level of diuron. A 3-generation reproduction sub-study was performed and the methods and results were included in 7. Reproduction Studies)

In two supplementary studies, rats (20/sex/dose) were feed diets containing 0, 250 or 2500 ppm for 15 (Diuron III) or 23 months (Diuron II).

Results
There was a high mortality observed in all groups. In males at 250 and 2500 ppm within the first 14 weeks (4, 2, 2, 9 and 10 in the ascending dose order), and later in control males during the second year (16/35, or total 27/35) which might be attributable to respiratory or other infections (see below). 17-48% males and 14-31% females of each group died by week 52; 60-88% males and 26-43% females died by week 90. Only 2, 9, 10, 5 and 1 males, and 19, 5, 17, 7 and 15 females respectively at 0, 25, 125, 250 and 2500 ppm survived to the end of study. Growth depression occurred for males and females at 250 ppm (up to 9% and 7% lower in body weight) and more severe at 2500 ppm (up to 10% and 18%). Food consumption was similar in all groups.

Rats at 250 and 2500 ppm, in particular females, showed on occasions reduced haemoglobin level and slightly lower RBC counts. The statistical significance was not achieved for these data, at least partially due to the low n values (n = 3-5). However, the study author claimed that the data for males were all within normal limits (historical data not present). An abnormal blood pigment was detected in males and females at 125 ppm and over from 9 to 16 months, but oxyhaemoglobin bands were normal. Urinalysis showed normal levels of sugar and protein.

Changes in haematology (n = 3-5)

Dose (ppm)		male					female				
		0	**25**	**125**	**250**	**2500**	**0**	**25**	**125**	**250**	**2500**
RBC, x 10^6/□L	12 M	8.1	7.6	7.3	7.4	6.7	6.9	6.1	6.6	6.0	5.8
	22 M	7.4	7.0	7.3	7.0	6.8	6.2	7.0	6.3	5.5	5.5
Haemoglobin, g/L	12 M	17.2	17.8	17.1	17.2	16.3	16.4	15.6	17.1	15.0	15.6
	22 M	16.9	16.5	16.0	16.0	15.5	15.6	16.8	15.7	14.4	14.9
Haematocrit %	12 M	53	54	53	53	52	48	45	49	45	46
	24 M	52	49	50	49	49	46	50	47	41	44

Organ weights were comparable across all groups. Splenic erythropoiesis was increased at 2500 ppm, but varied at other dose levels. The marrow fat content showed no consistent alteration, partially due to that either sternal or femoral marrows were taken from different groups. Although there was a questionable high value for male control (1/6 rat has a severe acute bronchopneumonia), the marrow M/E ratio was nevertheless lower in males at 2500 ppm and in females at 125 ppm and above. Microscopic changes in the bone marrow and spleen suggested increased turnover of RBCs. The haemosiderin content of the spleen was increased in both sexes at 250 and 2500 ppm, as well as in females at 125 ppm. Haemosiderin pigment was also found in the liver Kupffer cells of females at 2500 ppm

Histopathological findings in the spleen and bone marrows (n = 10/sex)

Dose (ppm)	male					female				
	0	**25**	**125**	**250**	**2500**	**0**	**25**	**125**	**250**	**2500**
Splenic erythropoiesis	2.7	2.3	1.7	1.8	3.4	2.7	2.7	3.7	2.5	3.2
Splenic haemosiderin	1.2	0.6	1.0	1.8	3.4	2.3	2.5	3.3	3.1	3.5
Marrow fat, %	19	27	21	29	4	20	12	24	19	19
Marrow myeloid / erythroid precursors ratio (M/E)	3.7	2.7	2.8	2.4	1.9	2.6	3.0	2.0	1.9	1.7

Atypical epithelial hyperplasia of the urinary bladder was observed in 3 males and 1 females at 2500 ppm, as well as in a male at each of control and 125 ppm, which, the study author argued, might be associated with an enzootic bladder infestation by the parasite Trichossomoides crassicauda.

At terminal sacrifice, every rat showed respiratory infection of some degree, corresponding to viral chronic murine pneumonia. Analysis of tissue levels of diuron at sacrifice indicated that with increasing dose levels, tissues tended to increase in diuron content, roughly in proportion to the dietary levels. A similar proportion of excretion was also seen in urine and faeces. No accumulation in the body was suggested.

Residues of diuron in tissues and excreta (ug/g tissue)

Dose (ppm)	**25**	**125**	**250**	**2500**
Muscle	0.19	0.58	0.65	8.9
Fat	0.56	2.3	3.6	51
Liver	0.72	4.3	4.5	49
Kidney	1.2	6.3	6.7	42
Spleen	0.77	2.1	3.6	17
Blood	0.41	2.8	2.6	20
Urine	6.3	42	49	492
Faeces	1.0	27	32	204

The two supplementary studies Diuron II and Diuron III were designed to determine the contribution of diuron to the high mortality seen in the main study. High mortality was again observed in Diuron II (13

and 13 males, and 9 and 14 females at 0 and 2500 ppm respectively for 23 months) and in Diuron III (6, 13 and 12 males, and 8, 7 and 10 females at 0, 250 and 2500 ppm respectively for 15 months), which was attributed, by the study, authors to respiratory and other infectious diseases. A slight growth depression was noted in rats at 2500 ppm (5-10% lower in body weight). Significant decreases in RBC counts and haemoglobin occurred at 2500 ppm, accompanied by an abnormal blood pigment and increased spleen weight. Histological examinations revealed changes in bone marrow and spleen in both sexes at 2500 ppm, and to a lesser degree in females at 250 ppm.

The quality of the study was affected by respiratory infection and high mortality of the animals, and is hence not considered to be suitable for regulatory purposes, and a NOAL is not established from this study.

Dogs

Hoffmann K & Schilde B (1985). Diuron-chronic toxicity to dogs after oral administration (12-month feeding study). Lab: Bayer Ag, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Report No: 13325. Study Duration: February 1982 – February 1983. GLP: yes.
&
Van Pelt CS & Hoffman K (1993). Supplement to diuron chronic toxicity to dogs after oral administration (12-month feeding study). Lab: Bayer Ag, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Report No: DIUR/TOX 10.

Methods
Beagle dogs (6/sex/dose) received 0, 50, 300 or 1800 ppm of diuron in the daily diet for 12 months. The dogs were supplied by F Winkelmann, D-4799 Borchen, 20-27 weeks old, and weighed 6.2-9.9 kg at the beginning of the study. Dietary mixtures (ssniff HH Sole Diet) with the test substance (98.2%, Batch no: 232 114 156) were prepared daily, and the concentration, homogeneity and stability of the test substance were analysed.

The dogs were observed daily for behaviour and appearance, and food consumption was measured daily and body weight weekly. The reflex tests (pupil, corneal, patellar tension, stretch, righting and bending), ophthalmoscopic, haematological (Appendix V, including blood sedimentation rate and Heinz bodies), clinical chemical (Appendix V, including glutamate dehydrogenase and serum protein electrophoresis, and excluding albumin, globulin, GGT, LDH, phosphorus and triglycerides) and urine examinations (Appendix V) took place on Weeks –2, 6, 13, 26, 39 and 52/53. At termination, organ weights (adrenals, brain, heart, kidneys, liver, lungs, ovaries, pancreas, prostate, spleen, thyroids, testes from all dogs), gross- (all organs/tissues from all groups) and histo-pathology (Appendix V, liver, spleen, kidneys and bone marrow from all groups, and other tissues including parotis; excluding skin, salivary gland, seminal vesicle, skin, spinal cord and vagina, from 0 and 1800 ppm groups) were examined.

Results
There were no deaths, no treatment-related clinical signs, no changes in reflex tests and no ophthalmoscopic findings. Slightly reduced food consumption in females and lower body weight gains (M: 2.8 *vs* 3.7 kg; F: 2.6 *vs* 3.7 kg) were observed at 1800 ppm.

Signs of anaemia occurred at 1800 ppm, characterised by a reduction in haemoglobin concentration and erythrocyte count, a rise in MCV, slight polychromasia and anisocytosis, an increased incidence of anulocytes, polychromatic and oxiphile normoblasts, Howell-Jolly bodies, and great quantities of Heinz' inclusion bodies in the erythrocytes. As the consequence, reticulocyte, leucocyte and platelet counts were increased, and the highly reactive fat-deficient bone marrow occurred with increased siderin content.

Changes in haematology and serum proteins at week 52
(Data presented as both sexes combined, n = 12)

Dose (ppm)	**0**	**50**	**300**	**1800**
RBC, 10^{12}/L	6.77	6.74	6.64	5.94**
Haemoglobin, g/L	160	160	158	142**
Haematocrit, L/L	0.46	0.46	0.46	0.42

MCV, fl	67.9	68.3	69.2	72.3**
MCHC, g/dl	350	350	346*	334**
Platelet, 10^9/L	238	230	268	359**
Leucocytes, 10^9/L	10.4	6.9	10.0	38.3**
Reticulocytes, %o	13.8	13.9	12.1	17.4**
Heinz bodies, %o	2.8	2.2	4.9	427
□1-globulin, %	6.56	6.40	6.20	5.97
□2-globulin, %	8.98	8.89	8.78	6.49**
□-globulin, %	18.4	18.4	18.5	19.5

*** p<0.05; ** p<0.01.**

Serum protein electrophoresis revealed changes in composition of the blood proteins, including a slight fall in alpha-1 and alpha-2 globulin proportions, and a corresponding increase in the beta globulin proportion, although the serum total protein content was unchanged. There were no treatment-related, toxicological significant findings in clinical chemistry and urinalyses.

At 1800 ppm, absolute and relative liver and spleen weights were increased. Gross findings in this group included distinct lobulation of liver with uneven surface in 1 male and 3 female dogs and dark bile or bile with black dust-like pigment in 3 females, enlarged spleen in 1 male and 1 female, and the spleen with dark and blunt-edge in 5 females, dark brown renal cortex in 2 males and 1 female, dark red to brown bone marrow in 4 males and 6 females. Histopathology detected increased incidences of haemosiderin deposition (iron-containing pigments) in Kupffer stellate cells of the liver of almost all dogs (11/12) and in reactive fat-deficient bone marrow with increased iron content was found in 8/12 dogs at 1800 ppm, in the red pulp of spleen (9/12 and 8/12 respectively) and in the basal region of the epithelial cells near the nucleus in the proximal tubule contorti of kidneys of dogs at 300 and 1800 ppm (6/12 and 10/12 respectively, appearing as golden brown crystalloid pigment). Testicle weights were also slightly higher at 1800 ppm without corresponding microscopic changes.

Organ weight changes (n = 6/sex)

	male				**female**			
Dose (ppm)	**0**	**50**	**300**	**1800**	**0**	**50**	**300**	**1800**
Liver, g	437	417	476	598	400	377	394	431
%	3.63	3.48	4.01	5.23	3.61	3.32	3.64	4.30
Spleen, g	50.8	51.3	64.2	74.2	43.3	33.3	38.8	63.0
%	0.42	0.42	0.54	0.64	0.39	0.29	0.35	0.61
Testes, g	19.4	19.1	21.9	24.3				
%	0.16	0.16	0.18	0.21*				

*** p<0.05.**

The study author argued that although pathological study of the spinal cord was not included in the study, no clinical evidence from reflex tests, or abnormality in the brain and sciatic nerve suggested any disorder in the nervous system. Tissues from low- and mid-dose groups, other than liver, spleen, kidneys and bone marrow, were not examined microscopically, since no treatment related gross findings in these tissues.

The NOEL was 50 ppm (approximate 1.25 mg/kg bw/day) based on haemosiderin deposition in the spleen and kidneys at higher doses.

Hodge H C & Downs W L (1964). Chronic feeding studies of diuron in dogs. Department of Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA. Report-no. 51485. Study Duration: November 1960 – November 1962. GLP/QA: no. Published: no.

Methods

Beagle dogs (3/sex/dose) received 0, 25, 125, 250 and 1250/2500 ppm of diuron (as a 80% wettable powder) in the daily diet for 24 months. The dose levels were determined by a pilot study and adjusted at the beginning of the study. The high dose group received 2500 ppm for 2 weeks initially, then ceased dosing due to food refusal and body weight loss, and reduced to 1250 ppm after a 3-week

reconditioning period. Dietary mixtures containing the test substance (Code T 7111 3D, received from E I DuPont de Nemours and Company, Inc. Wilmington, Delaware) were prepared weekly.

The dogs were observed regularly for general clinical condition and appetite, and weekly for food consumption and body weight. Blood samples were collected pre-test, at 2-week after start, and every 3 months thereafter for haematology (RBC count, haemoglobin and haematocrit), and urine samples monthly for sugar and protein. At termination, organ weights (liver, kidneys, lungs, brain, heart, spleen, testes), gross- (all organs) and histo-pathology (brain, lungs, heart, liver, spleen, kidneys, adrenals, gonads, stomach, large and small intestines, urinary bladder, bone marrow, muscle and thyroid) were examined. Concentrations of diuron in tissues (muscle, fat, liver, kidney, spleen, blood) were analysed at termination and residue in urine and faces was tested at 6 weeks prior to termination.

Results

The highest residue level was detected in the liver. With considerable variability from tissue to tissue, all other tissue from dogs of the same group had roughly comparable levels. With increasing dose levels, tissues tended to increase in diuron content, roughly in proportion to the dietary levels. A similar proportion was also seen excretion in urine and faeces. No accumulation in the body was suggested.

Residues of Diuron in Tissues and Excreta (ug/g tissue, n = 6)

Dose (ppm)	**25**	**125**	**250**	**1250**
Muscle	0.25	0.41	0.90	5.1
Fat	0.54	2.7	5.7	33
Liver	3.7	9.8	16	56
Kidney	0.83	4.3	5.4	33
Spleen	0.36	1.1	1.9	9.7
Blood	0.37	1.3	2.2	14
Urine	6.3	41	42	307
Faeces	7.9	41	68	308

One female at 25 ppm and one male at 1250 ppm were sacrificed at Week 8 or 76 due to heart disease and necrotic strangulated hernia respectively. Body weight loss was observed at 1250 ppm, particularly severe and persistent in 2 males and 1 female (up to 4 kg during the 2 years).

Significantly decreased RBC counts, haemoglobin and haematocrit values were noted at 1250 ppm from 3 months onwards, and to a less extent at 125 and 250 ppm. Spectral analysis of haemoglobin revealed abnormal pigments in all dogs at 250 and 1250 ppm, and in 2/3 males at 125 ppm. Urine analysis for protein or sugar did not show abnormalities in any groups.

Changes in haematology (n = 3/sex)

Dose (ppm)		**male**					**female**				
		0	**25**	**125**	**250**	**1250**	**0**	**25**	**125**	**250**	**1250**
RBC, 10^6/□L	105 d	7.6	7.0	6.8	6.6	5.3**	6.8	6.7	7.1	5.6	4.8*
	1 y	7.3	6.8	6.5	5.8	5.1*	6.4	7.1	6.9	5.3	4.4**
	2 y	6.9	6.9	6.4	5.7*	4.9*	6.3	6.7	6.4	5.5	4.5**
Haemoglobin, g/L	105 d	20.6	17.7	17.8	17.1	15.8*	18.0	17.9	18.6	16.1	13.7**
	1 y	21.5	19.0	19.4	17.3	15.0**	18.7	19.6	19.4	17.3	14.3**
	2 y	20.0	18.5	18.5	17.3	15.3*	18.9	19.0	17.5	16.5	13.7**

***p<0.05; **p<0.01 by *t-test*.** **- : no data.**

Bone marrow showed a statistical increase in erythrogenic activity/erythroid hyperplasia, and moderate reduction in marrow fat at 1250 ppm, and the hepatic Kupffer cells had increased brown

pigments, presumably haemosiderin. Haemolytic anaemia and secondary stimulation of erythropoietic activity were suggested.

Absolute and relative liver weights were higher in dogs at 1250 ppm, but slightly increased relative weights for other organs of this group might only reflect the lower body weight. No treatment-related neoplasia findings were reported.

The NOEL was 25 ppm (approximate 0.6 mg/kg bw/day) due to reduced RBC and haemoglobin levels and abnormal blood pigments at higher doses.

7. REPRODUCTION STUDIES

Rats

Cook, JC (1990). Reproductive and fertility effects with diuron (IN 14740): Multigenerational reproduction study in rats. Report no: HLR 560-90. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714, USA. Medical Research Project No: 8670-001. Duration of study: April 1989 – January 1990. Guidelines: US EPA 83-4. GLP: yes. Published: no.

Methods
In a two-generation reproduction study, rats (P, 30/sex/dose) received 0, 10, 250 or 1750 ppm of diuron in the daily diet for 73 days, and then bred within the dose groups to produce F1 litters. At weaning on Day 21, 30 F1 rats/sex/dose were randomly selected, and at least 105 days after weaning, the F1 rats were bred to produce F2 litters. For both generations, administration of the test substance continued during breeding, gestation and lactation. The P rats (Crl:CD BR) were supplied by Charles River Laboratories Inc, Raleigh, North Carolina, and were approximately 6 weeks, 120-163 g for males and 83-139 g for females at the beginning. The test substance (Purity 97.1%) was received from Du Pont Agricultural Products, Wilmington, Delaware 19898. Food consumption and body weight were measured weekly.

On day 4 postpartum, the litters were culled randomly to 8 (4/sex if possible). Pups were counted, weighed and examined on the day after delivery (Day 0), after culling on Days 4, 7, 14 and 21 postpartum. Offspring that died during the lactation period were necropsied (except 4 pups due to technical error).

All parental generation rats were sacrificed for gross examinations after litter production. The testes were weighted, and the reproductive tissues (testes, epididymides, prostate, seminal vesicles, coagulating glands, pituitary, and ovaries, uterus, vagina and pituitary) from 0 and 1725 ppm groups, and gross lesions from all groups were collected for examination microscopically. From F1 weanlings not selected to continue to the next generation and from surviving F2 weanlings, approximately 20/sex/group were selected for gross post-mortem examination.

Results
The purity, homogeneity and stability of diuron in the diet were confirmed by analysis. The calculated daily intakes of diuron are listed below.

Daily intake of diuron (mg/kg bw/day)

	male			**female**		
Dose (ppm)	**10**	**25**	**1750**	**10**	**25**	**1750**
P1 (premating)	0.6	14.8	101	0.7	18.5	131
P1 females (gestation)				0.7	18.2	128
F1 (premating)	0.8	18.9	139	0.9	22.1	157
F1 females (gestation)				0.7	16.4	116

At 1750 ppm, mean body weight gains were lower in P and F1 male (17-18% lower than control), in P and F1 female (28% and 14% lower) during premating, and in P and F1 female (7% and 19% lower) during gestation. However, P and F1 females at 1750 ppm had less body weight loss or gained more weight than control during lactation. Mean food consumption at 1750 ppm was also lower in P and F1 males (8% and 12% lower), P and F1 females during premating (6% and 11% lower) and gestation (10% and 15% lower).

No treatment-related effects were detected on reproduction parameters (mating and fertility indices) of P and F1 parental rats. Relative (but not absolute) weights of testis in P and F1 males at 1750 ppm were significantly higher, probably due to lower final body weights of the groups. Large spleens of P (4/30) and F1 females (7/30) were observed at 1750 ppm, microscopically correlated with congestion.

Litter size and pup survival rates were normal in all groups. Mean litter weights and male and female pup weights of F1 (up to 17-19% lower) and F2 litters (up to 18-19% lower) were lower at 1750 ppm. No treatment related gross abnormalities were observed in F1 or F2 weanlings and in unscheduled sacrificed pups.

The NOEL for general toxicity was 250 ppm (approximate 25 mg/kg bw/day) based on lower food consumption and body weight gain in P1 and F1 adults at the higher dose. The NOEL for reproduction toxicity was 1750 ppm, the highest dose in this study. The NOEL for foetal toxicity was 250 ppm based on lower pup weights at the higher doses.

Hodge H C & Downs W L (1964). Chronic feeding studies of diuron in rats. Department of Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA. Report No: 51486. MRID No: 17764. Study Duration: June 1960 – June 1962. Guidelines: no. GLP: no. Published: no.

Methods
Rats (35/sex/dose) received diuron (as a wettable powder of 80% diuron) at 0, 25, 125, 250 or 2500 ppm in the daily diet for 24 months. The dose levels were determined by a pilot study. The dietary mixtures (Purina Fox Chow Meal) with the test substance (Code T 71113D, received from E I DuPont de Nemours and Company, Inc. Wilmington, Delaware) were prepared weekly. The observations for general toxicity were included in Chronic Studies. A 3-generation reproduction sub-study was made by selecting 16 females and 8 males from the 0 and 125 ppm groups (P) for the first mating (F1a) at 100 days of age, and the second mating (F1b) 10 days after weaning of the first litter. Selected 16 females and 8 males from the second litters of each dose group (F1b) continued on their respective diets from weaning and mated (F2a) at 100 days of age and again (F2b) 10 days after weaning the first litter. F3 generation was produced from F2b similarly. 10 males and 10 females from the two F3 litters (F3a & F3b) of each dose group were sacrificed at the age of 21 days, and studied histologically for abnormalities. In breading the male animals are rotated each group weekly. Records are made of matings, number of pregnancies, numbers of pup in the litter at 1, 5 and 21 days. Litters were reduced to 10 pups/litter on day 5.

Results
Reproductive performance was comparable for treated rats (125 ppm) and control. Females of F1b and F3b litters, both sexes of F2b and F3a litters showed weight depression after 4 and/or 10 weeks at 125 ppm which persisted even though the rats were then receiving the basal diet. F3b rats sacrificed at weaning exhibited no treatment-related changes in haematology, organ weights and histology. The second three-generation reproductive study did not reveal growth retardation in litters.

8. DEVELOPMENTAL STUDIES

8.1 Rats

Dearlove G E (1986a). Developmental toxicity study of H-16035 administered by gavage to rats (with Supplement 1). Lab: Argus Research Laboratories, Inc, Horsham, Pennsylvania 19044 USA. Study Duration: December 1985 – January 1986. Report No: HLO 410-86. GLP: yes.

Methods

Diuron was gavage at 0, 16, 80 or 400 mg/kg bw/day (5 mL/kg/day) once daily to female rats (25/dose) on presumed gestation Days 6 – 15. The rats (Crl:COBS CD (SD)BR) were supplied by Kingston, New York facility of Charles River Breading Laboratories, Inc, and were 234 to 294 g at the beginning of the study. The suspension of the test substance (Batch No: B-104-012-A/B/C/D, IN-14740-146, H-16035, Purity: 99.0%, provided by E I DuPont de Nemours and Company, Inc. and suspension in aqueous 0.5% hydroxypropyl methylcellulose) were prepared and used for the following 2-4 days, and dosing concentrations were determined by analyses. The rats were observed daily for clinical signs, abortion and/or death. Body weight was recorded on Days 0, 6, 9, 10, 12, 16 and 20, and food consumption on Days 0, 6, 10, 16 and 20. On Day 20 of presumed gestation, the rats were sacrificed with carbon dioxide and necropsied. The liver was weighed. The uterus was examined for pregnancy, number and placement of implantations, early and late resorptions, and live and dead foetuses. Corpora lutea in each ovary were counted. Each foetus was weighed, sexed and examined for external alteration. Approximate one-half of all live foetuses in each litter were killed and examined for visceral alteration, and all foetuses were examined for skeletal alteration.

Results

22-23 out of 25 rats per group were pregnant. Pregnant rats at 80 and 400 mg/kg bw/day had significantly lower food consumption during the entire dosing period, in particular at the early stage of dosing. Consequently, rats in these groups exhibited body weight loss (4% and 10% respectively) during days 6-9, and less weight gain at the latter stage of dosing. A significant rebound effect in food consumption and body weight change of these groups occurred after completion of dosing (days 16-20), although body weight was still significantly lower at 400 mg/kg bw/day by the end of the study. Absolute liver weights were similar across groups, and relative liver weight (to body) was significantly higher at 400 mg/kg bw/day, which might reflect lower body weight of this group.

Body weight and food consumption of dams (n = 25)

	Body weight (g)					**Food consumption (g/day)**			
mg/kg bw/day	**0**	**16**	**80**	**400**		**0**	**16**	**80**	**400**
Day 6	300	299	298	298	Days 0-6	21.0	21.3	20.5	21.2
Day 9	304	299	286**	268**	Days 6-10	20.8	20.1	14.6##	7.6##
Day 12	317	314	290**	265**					
Day 16	339	337	312**	285**	Days 10-16	22.8	23.0	17.0@@	12.3@@
Day 20	396	397	380	364**	Days 16-20	24.7	26.1@	27.1@@	26.9@@

** p < 0.01 by *One-way ANOVA*. ## p < 0.01 by *Dunnett's Test*.
@@ p < 0.01 by *Mann-Whitney U* Test.

There were no treatment-related effects on the rate of pregnancy, litter size, resorptions or foetal sex ratio. Average foetal body weights were significantly lower (9% lower) for litters at 400 mg/kg bw/day, compared with control. Skeletal examinations revealed a higher incidence of foetuses with delayed ossification of the vertebrae and sternum (sternal centres) at 400 mg/kg bw/day.

Main fetal skeletal alterations (no of fetals / no of litters)

mg/kg bw/day	**0**	**16**	**80**	**400**
Vertebrae, thoracic, centra, bifid	1/1	2/1	4/2	10/7*
asymmetric			1/1	1/1
unilateral ossification			1/1	1/1
not ossified				4/3
Incompletely ossified				1/1
centrum and arch, hemivertebra			1/1	
lumbar, arches, incompletely ossified				2/2
sacrals, not ossified		1/1		2/1

caudals, not ossified		2/2		9/1
Sternebrae, not ossified	3/3	3/3	2/2	14/5
incompletely ossified	3/3	5/3	1/1	16/9*
Xiphoid, not ossified	1/1	3/3	1/1	13/4
Pelvis, pubes and/or ischia, not ossified	1/1	2/2		12/2

* $p < 0.05$, compared with control, by Jonckheere's test and Mann-Whitney U or Fisher's exact test

The NOEL for maternal toxicity was 16 mg/kg bw/day based on the lower food consumption and body weight gain at higher doses. The NOEL for developmental toxicity was 80 mg/kg bw/day based on lower pup weight and developmental retardation of skeleton at the next higher dose.

Rabbits

Dearlove GE (1986b). Developmental toxicity study of H-16035 administered by gavage to New Zealand White rabbits. Lab: Argus research laboratories, Inc., 935 Horsham Road, Horsham, Pennsylvania 19044 USA. Report-no. HLO 322-86. Study Duration: November 1985 – January 1986. GLP: yes.

Methods
Artificially inseminated female New Zealand White rabbits (23-25/dose) received daily doses of diuron at 0, 2, 10 or 50 mg/kg bw/day (5 mL/kg/day) by gavage on presumed gestation Days 7-19. The rabbits (Hra:SPF) were from Hazleton Research Animals, Denver, Pennsylvania, and the test substance (IN-14740-146, H-16035, Purity: 99.0%, provided by E I DuPont de Nemours and Company, Inc.) was made as a suspension in 0.5% aqueous hydroxypropyl methylcellulose. The rabbits were observed daily for clinical signs, abortion and/or death. Body weight was recorded on Days 0, 7, 10, 13, 16, 20, 24 and 29, and similarly for food consumption. On Day 29 of presumed gestation, the rabbits were sacrificed with T-61 Euthanasia Solution and necropsied. The liver was weighed. The uterus was examined for number and placement of implantations, early and late resorptions, and live and dead foetuses. Corpora lutea in each ovary were counted. Each foetus was weighed and examined for external alteration. The foetuses were examined for visceral and skeletal alterations.

Results
21-23 rabbits each group were pregnant. Pregnant rabbits at 50 mg/kg bw/day had significantly lower food consumption during the entire dosing period, in particular at the later stage of dosing. Consequently, rats in these groups exhibited lower body weight gain or body weight loss. A rebound effect in food consumption and body weight change occurred in this group after completion of dosing (Days 20-29).

Body weight gains and food consumption (n = 21-23)

	Body weight gain (g)		**Food consumption (g/day)**	
mg/kg bw/day	**0**	**50**	**0**	**50**
Days 7-10	0.03	0.04	177	168
Days 10-13	0.06	0.02*	166	146
Days 13-16	0.04	- 0.04**	150	97**
Days 16-20	0	- 0.06	143	90**
Days 7-20	0.14	- 0.04**	157	123**
Days 0-29	0.32	0.34	148	135

* $p < 0.05$, ** $p < 0.01$ by Dunnett's Test.

One rabbit at 50 mg/kg bw/day aborted on Day 26 of gestation. No clinical signs or gross lesions were attributed to treatment. Liver weights were similar cross groups. The mean foetal weight, number of corpora lutea, implantation sites, live foetuses and incidences of resorption and/or foetal death were comparable across all groups. No external, soft tissue or skeletal alterations demonstrated either a significant or dose-related incidence, as compared with control.

The NOEL for maternal toxicity was 10 mg/kg bw/day based on the lower food consumption and body weight gain at the higher dose. The NOEL for developmental toxicity was 50 mg/kg bw/day, the highest dose tested in the study.

9. GENOTOXICITY STUDIES

A summary of submitted and published findings of genotoxicity studies is tabulated below.

Summary of *in vitro* Genotoxicity Studies

Assay	Bacterial strain or Cell type	Conc. or Dose	Batch / Purity	Metab. Act.	Result	Reference
Gene Mutation						
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA1535 TA 1537	Test 1: 20-2500 μg/plate; Test 2: 125-2000 μg/plate	232 114 080 / 98.8%	+, - +, - +, - +, -	-, - -, - -, - -, -	Herbold (1984a)
	S. typhimurium TA 97 TA 98 TA 100 TA1535	S9: 0.5-10 μg/plate; + S9: 10-250 μg/plate	*H-15580* / 98.2%	+, - +, - +, - +, -	-, - -, - -, - -, -	Arce (1984/1987)
	S. typhimurium TA 97 TA 98 TA 100 TA1535	S9: 10-750 μg/plate; + S9: 50-5000 μg/plate in DMSO	IN-15926-6 / 99.9%	+, - +, - +, - +, -	-, - -, - -, - -, -	Reynolds (1992a)
	S. typhimurium TA 97 TA 98 TA 100 TA1535	± S9: 10-5000 μg/plate in DMSO	DPX-12402-46; IN-12402-46 / 99.8%	+, - +, - +, - +, -	-, - -, - -, - -, -	Reynolds (1992b)
	S. typhimurium TA 97 TA 98 TA 100 TA1535	± S9: 10-5000 μg/plate in DMSO	IN-12894-5 / 99.8%	+, - +, - +, - +, -	-, - -, - -, - -, -	Reynolds (1992c)
	S. typhimurium TA 97a TA 98 TA 100 TA1535 *Escherichia. Coli* WP2uvrA (pKM101)	± S9: 10-5000 μg/plate	DPX-14740-194 (Karmex DF) / 81.3%	+, - +, - +, - +, - +,-	-, - -, - -, - -, - -,-	Bentley (1995a)
	S. typhimurium TA 97a TA 98 TA 100 TA1535 *Escherichia. Coli* WP2uvrA (pKM101)	± S9: 10-5000 μg/plate	DPX-14740-200 (Karmex 500 SC, Lot 057-94-2285) / 41.8%	+, - +, - +, - +, - +,-	-, - -, - -, - -, - -,-	Bentley (1995b)
	S. typhimurium TA 98 TA 100 TA1535 TA 1537 TA 1538	± S9: 20-1600 μg/plate	551/6/97; D09/017/12/97 / 98.8%	+, - +, - +, - +, - +,-	-, - -, - -, - -, - -,-	Kamath (1998)
	S. typhimurium TA 98 TA 100 TA1535 TA 1538	± S9: 0.1-1000 μg/plate	Diuron technical active (no details)	+, - +, - +, - +,-	-, - -, - -, - -,-	Kiss (1986)
	S. typhimurium TA 98 TA 100 TA102 TA1535 TA 1537	± S9: 9-150 μg/plate	D9SP074 / 97%	+, - +, - +, - +,- +,-	-, - -, - -, - -,- -,-	Rajwani (2000).
	Escherichia. coli	± S9: 312-5000 μg/plate	232 114 080 / 98.8%	+, -	-, -	Herbold (1984b)

Mutagesis at HPRT locus	CHO cells	± S9: 10-1250 nM	Lot T50906, Batch 04 / 98.2%	+,-	-,-	Ullman & Choy (1985)
	CHO cells	± S9: 180-310 μg/mL	551/6/97; D09/017/12/ 97 / 98.8%	+,-	-,-	Shivaram (1998)
DNA Damage and Repair						
Unscheduled DNA synthesis	Primary rat hepatocytes	0.001-20 mM	98.2%		+/-	Arce (1985)*
Chromosomal Effect Assays						
Chromosomal Aberration	CHO-K1 cells	0 (DMSO), 90, 180, 360 μg/mL	551/6/97; D09/017/12/ 97 / 98.8%	+, -	-,-	Shivaram (1999)
Sister Chromatid Exchange	CHO-K1 cells	0 (dimethyl sulfoxide), 0.001 – 0.1 □g/mL	Diuron technical active (no details)	+, -	-, -	Kiss (1986)
Cytogenetic study	Human lymphocytes	500 μg/mL; 1000 μg/mL	232114123/ 98.7%	+,-	+, +	Herbold (1989)*

Results (+, positive; -, negative or +/-, equivocal) are expressed relative to the presence (+) or absence (-) of metabolic activation.
*Studies with positive or equivocal results are assessed with detailed information as the following).

Summary of *in vivo* Genotoxicity Studies

Assay	Species (Strain)	Dose	Batch/Purity	Result	Reference
Gene Mutation					
Dominant lethal mutation	Mice (Bor:NMRI, SPF Han) (germ cells)	Test 1: 2000 mg/kg Test 2: 2500 mg/kg Single oral dose in 0.5% cremophor.	232114080 / 98.8% 232355721 / 98.1%	+/-	Herbold (1985)*
Chromosomal Effect Assays					
Cytogenetic assay	Mouse (NMRI) (germ cells)	0 (2% cremophor), 500-5000 mg/kg bw, killed at 6, 24 or 48 h.	232114123 / 98.4%	-	Volkner (1988)
Chromosomal aberration	Rat, bone marrow cells	0 (corn oil), 50-5000 mg/kg bw, oral, killed at 6, 24 or 48 h.	Lot: T50906, Batch: 04 / 98.2%	+/-	Ullman (1985)/Cox (1997)*
Chromosomal Aberration	Chinese hamster (bone marrow cells)	0 (cremophor), 500-5000 mg/kg bw, oral, killed at 6, 24 or 48 h.	232114123 / 98.4%	-	Volkner (1987a)
Sister Chromatid Exchange	Chinese hamster (marrow cells)	0 (2% cremophor), 500-5000 mg/kg bw, killed at 24 h.	232114123 / 98.4%	-	Volkner (1987b)
Micronucleus	Mice (Bor:NMRI, SPF Han) (marrow cells)	0 (0.5% cremophor), 2500 mg/kg bw by po, killed at 24, 48 or 72 h.	232114080/98 .8%	-	Herbold (1983)
	Mice (Hsd/Win: NMRI) (marrow cells)	0 (0.5% cremophor), 700 mg/kg bw by ip, killed at 16, 24 or 48 h.	232455681 / 98.1%	-	Herbold (1998)
	Swiss albino mice (marrow cells)	0 (DMSO), 85, 170, 340 mg/kg bw, ip. killed at 30 48 or 72 h.	Diuron from Sigma.	+	Agrawal et al (1996)*
	NMRI outbred mice (marrow cells)	0 (oleum helianthi), 300, 314, 602, 628 mg/kg bw, killed at 24, 48 or 72 h.	Diuron technical active (no details).	-	Kiss (1986)
DNA Damage and Repair					
Unscheduled DNA synthesis (urinary bladder epithelial cells)	Female rats (BOR:WISW)	0, 25, 250, 2500 ppm in the diet, killed at 24 h.	No information.	+	Klein (1986)*

Results are expressed as +, positive; -, negative; +/-, equivocal.
*Studies with positive or equivocal results are assessed with detailed information as the following.

Klein W (1986). Induction of and effect on UDS by test substance diuron. Lab: Austrian Research Center Seibersdorf, Ges m b H A-2444, Seibersdorf. Bayer Study No: T 4021984. Study duration: October 1986. QA: yes.

Methods

Female rats were given diuron 0, 25, 250 or 2500 ppm (11, 104 or 687 mg/kg bw/day) or the positive control methyl methane sulphonate (MMS) 100 mg/kg bw in the daily diet (Altromin 1321) for 7 days. The rats (BOR:WISW) were supplied by Winkelmann Versuchstierzucht Ges m.b.H., D-4799 Borchen. At sacrifice, the urinary bladder was perfused, incised into small pieces and digested with 0.25% trypsin solution for 30 min x 3, and the cells were harvested from the suspensions. The cells were then incubated with 10 □Ci ^{3}H-thymidine in F12 medium for 2 hours at 37^{o}C, followed by washing, making slides and autoradiographic preparations. The number of silver grains per cell nucleus in 100 cells was counted microscopically, and the proportion of S phase cells was determined in collectives of 200 cells.

Results
Food consumption was reduced in rats at 2500 ppm. Induction of UDS was indicated by a significant increase in the mean silver grain count per cell, and in the number of cells with higher grain counts in 250 and 2500 ppm groups. There was also a dose-related increase in S phase cell proportions. Simultaneously, the extent of repair on damage to the DNA of the urinary bladder epithelial cells was reduced at 250 and 2500 ppm, suggesting reduced degree of DNA repair. Differentiated evaluation of the grain counts showed a significant increase only in cells with 3 silver grants in these groups.

Induction of UDS and degree of repair

Dose (ppm)	**Diuron**				**MMS**
	0	**25**	**250**	**2500**	
UDS (% control)	1.00	0.99	1.53*	1.85*	9.56*
S-phase cells (%)	0.17	0.42	1.33*	2.67*	2.25*
DNA repair (% control)	100	94	85*	81*	69*
No of cells with 3 silver grains	1	5	13*	13*	65*
with 4 silver grains	3	0	1	3	38*
with 5 silver grains	0	0	1	5	133*

* Significant by 2-sided t test.

As speculated by the author of the report, irritant effects on the bladder tissue through a concentration of test substance/metabolites in the urine may be indirectly contribute to a delay in DNA repair. Hence a direct genotoxic effect might not be concluded.

Herbold B (1985). Diuron: Dominant lethal test on the male mouse to evaluate for mutagenic effect. Lab: Bayer AG, Institute of Toxicology. Study No: T 6015947; T6016685. Study duration: July 1983 – March 1984.

Methods
In two independent tests, male mice (50/group) were given an oral dose of diuron (98.8% for Batch 232114080, and 98.1% for Batch 232355721) at 0 or 2000 mg/kg bw in Test 1, and 0 or 2500 mg/kg bw (2 sub-groups, 50 mice/sub-group) in Test 2. Following dosing, each male was mated for 12 periods, with an untreated female for 4 days in each period (Theoretically, all germ cells present in the testicles at the time of treatment could be used for insemination and fertilisation). No checking was made for vaginal plug. Approximately 14 days after the mid of each mating period, the females were sacrificed and examinations took place to determine the numbers of total, dead and live implants, live germ cells and corpora lutea, in order to establish pre- and post-implantation losses. The mice (Bor:NMRI, SPF Han) were supplied by F Winkelmann, Borchen, were 8-12 weeks old at the beginning of the study and feed with Altromin 1324. A total of 36 mating periods (12x3), and approximate 150 males and 1800 females (untreated) were used and evaluated in this study. No positive control was included in this study.

Results
No mortality or clinical signs were observed following dosing at 2000 mg/kg bw in Test 1. Somnolence occurred in mice after dosing at 2500 mg/kg bw in Test 2, and 9 mice died before mating.

The fertilisation rate was comparable between the treated and control groups, with the exception of that in Subgroup 1, Period 1 of Test 2, it was lower in the treated group compared to control (63% vs 86%). This was presumably attributable to acute toxicity of the substance. A higher rate of dead implantation was observed in Period 5 and 6 of Test 1 (dead implants/female: 1.4 *vs* 0.7, 1.8 *vs* 1.0 in control respectively). However, the majority of mice in the tests did not show any relevant indications of mutagenic effect induced by the test substance.

The test substance did not show an effect on fertilisation and implantation, which is consistent with observations in the reproduction study. Diuron was not considered to be genotoxic in the germ cells of male mice under the condition of the test.

Herbold BA (1989). In vitro cytogenetic study with human lymphocytes for the detection of induced clastogenic effects. Lab: Bayer AG. Study No: T0029478. Study Duration: April 1988 – February 1989. GLP/QA: yes.

Methods
Diuron (Batch No: 232114123, 98.7%) was evaluated for its clastogenic effects in human lymphocytes, at concentrations of 0 - 500 □g/mL (DMSO as the solvent) in the absence of rat S9 mix, and 250 – 1000 □g/mL in the presence of S9. Mitomycin C and cyclophosphamide were used as positive control without and with S9 mix respectively.

Results
Precipitation of the test substance occurred at 500 □g/mL and over. Concentration-dependent reduction of the mitotic index indicated a significant cytotoxic effect of the test substance in the absence and presence of S9 mix. The test substance induced concentration-related higher incidences of metaphase aberrations including or excluding gaps, and metaphases with exchanges, at tested concentrations from 125 □g/mL without S9 mix, and from 500 □g/mL with S9 mix.

Clastogenic findings [expressed as no (%)]

Diuron (□g/mL)	Test 1				Test 2			
	Mitotic nuclei	Aberration		Ex-change	Mitotic nuclei	Aberration		Ex-change
		+ gaps	- gaps			+ gaps	- gaps	
- S9								
0	145 (100)	18 (9)	0 (0)		168 (100)	13 (6.5)	4 (2)	
62.5	220 (152)	24 (12)	4 (2)		-	-	-	
125	123 (85)	34* (17)	11* (5.5)		121* (72)	12 (6)	2 (1)	
250	114* (79)	34* (17)	5* (2.5)		88* (52)	24* (12)	9 (4.5)	
500	-	-	-		60* (36)	36** (18)	17** (8.5)	1 (0.5)
MMC	169 (117)	83** (42)	52** (26)	7 (3.5)	113* (67)	60** (30)	35** (17.5)	4 (2.0)
+ S9								
0	270 (100)	16 (8)	9 (4.5)		196 (100)	9 (4.5)	3 (1.5)	
250	185** (68)	26 (13)	8 (4.0)		-	-	-	
500	129** (48)	30* (15)	13 (6.5)	2 (1)	137* (70)	14 (7)	4 (2.0)	
750	48**(18)	31* (16)	11 (5.5)	1 (0.5)	57* (29)	27** (14)	17** (8.5)	2 (1)
1000	-	-	-		18* (9)	17** (17)	7* (7.0)	2 (2)
CYCL	199** (74)	55** (28)	31**(15.5)	2 (1)	106* (54)	84** (42)	55** (28)	9**(4.5)

MMC: mitomycin C. CYCL: cyclophosphamide. * $p<0.05$; ** $p<0.01$ in Chi^2 test.

Diuron exhibited clastogenic effect at the concentration range tested in this study.

Arce GT (1985). Assessment of diuron in the in vitro unscheduled DNA synthesis assay in primary rat hepatocytes. Lab: El du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Lab Report No: 349-85. Study Duration: October 1984 – July 1985. GLP/QA: not stated.

Methods
Diuron (Lot No: T50906, Batch No: 04, Purity: 98.2%) was assessed for its effect on unscheduled DNA synthesis in primary rat hepatocytes. Freshly isolated hepatocytes from male rats in the labelled culture (^{3}H-thymidine) on slides were incubated with 0.001 – 20 mM of diuron (in DMSO) or the

positive control dimethylbenzanthracene (DMBA) for 18 hours. The slides were randomised, coded and scored for UDS activity. Incorporation of ^{3}H-thymidine into DNA was analysed by autoradiography. The net grain count is the difference between the cytoplasmic and nuclear grain counts.

Results
Precipitate was observed at 1-20 mM of diuron. Cytotoxicity was evident at 0.33 mM and above. Diuron at 0.33, 1 and 20 mM induced statistically significant increases in net grain counts, compared to control. However, the increase in the net grain counts with dose was attributed to a decrease in the cytoplasmic grain counts which was probably relevant to cytotoxicity, rather than an increase in the nuclear grain counts.

UDS in primary cultures of rat hepatocytes

Dose (mM)	**Teat 1**		**Test 2**	
	Average net nuclear grains	**mean nuclear grains – mean cytoplasmic grains**	**Average net nuclear grains**	**mean nuclear grains – mean cytoplasmic grains**
0	- 2.5	15.2-17.8	- 2.4	15.0-17.4
Diuron 0.001	- 1.0	10.0-11.0	- 2.1	14.0-16.1
0.010	- 1.3	13.7-15.0	0	16.3-16.2
0.100	0.1	12.4-12.3	- 2.0	15.4-17.4
0.33	2.0	11.2-9.2	0.1	13.9-13.8
1.00	1.7	9.9-8.2	1.4	12.4-10.9
20.0	1.6	10.3-8.7	1.6	13.1-11.6
AMBA 0.1	41.4		44.0	

Hence, the statistically significant increases in the net grain counts did not reflect test substance-induced UDS.

Ullman (1985)/Cox (1997). In vivo assay of diuron for chromosome aberrations in rat bone marrow cells. Lab: EI du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Lab Project ID: HLR #366-85.

Methods
A single dose of 0, 50, 500 or 5000 mg/kg bw of diuron (Lot No: T 50906, Batch No: 04, Purity: 98.2%, as a suspension in corn oil) or 20 mg/kg bw of cyclophosphamide as positive control was given to rat (5/sex/dose/time point, Crl:CD(SD)BR form Charles River, Kingston NY) by gavage. At sacrifice approximate 6, 24 and 48 h after dosing, bone marrow cells were harvested and slides were prepared and stained for cytogenetic analysis. 50 metaphase cells per rat were analysed for chromosomal aberration.

Results
Rats at 5000 mg/kg bw/day showed clinical signs including red, orange, or yellow-coloured discharge from the mouth, nose and/or eyes, reduced activity or depression, wet, stained perineum, laboured respiration, moribundity, diarrhoea, salivation and tremors, predominantly at 24 and/or 48 h after dosing. One of the moribund females was found dead 48 h after dosing. Diarrhoea was seen in some rats at 500 mg/kg bw/day, and red-stained neck fur was noted in 1 female at 50 mg/kg bw/day. Significant weight loss was seen in rats of 24 and 48 h groups at 5000 mg/kg bw/day, and in females of 48 h group at 500 mg/kg bw/day.

Significant mitotic index depression was seen in preparations from male rats at 5000 mg/kg bw at both 24 and 48 h sacrifices, indicating cytotoxicity. Small but significant increases in the percentage abnormal cells and aberrations per cell were seen at 5000 mg/kg bw at 48 h. A significant positive linear dose trend was also observed for these endpoints. However, the magnitude of increases in the aberration was relatively small and the mean values fall within the range of historical control data. The aberrations seen were mostly chromatid-type breaks, and no exchange figures were detected among diuron treated animals.

In vivo chromosomal aberration in rat bone marrow at 48 h (n = 5)

Dose (mg/kg bw/day)	Abnormal cells / group (%)		Cells with aberration (%)		No of aberrations / cells		No of mitoses / 500 cells	
	male	female	male	female	male	female	male	female
0	0.0	0.0	0	0	0.000	0.000	11.4	9.8
50	0.0	0.4	0	0	0.000	0.004	8.4	9.2
500	0.8	0.4	0	0.4	0.008	0.008	10.6	10.0
5000	1.2	0.5	0.4	0	0.016	0.005	4.6**	6.5
5000 (m+f)	0.9*		0.2		0.011*		5.4***	
Historical control[#]	0-2.6	0.2.0	-	-	0-0.023	0-0.060	-	-

*p<0.05, **p<0.01, *** p<0.001 by *Student* t-test.
[#] Compiled from 14 studies performed at Haskell Laboratory and/or contract laboratories from 1983-1986.

Diuron was considered negative in this *in vivo* assay.

Agrawal RC, Kumar S & Mehrotra NK (1996). Micronucleus induction by diuron in mouse bone marrow. Toxicology Letters 89: 1-4.

Methods
A single dose of 0 (DMSO), 85, 170 or 340 mg/kg bw of diuron (Sigma), or 20 mg/kg bw of cyclophosphamide as positive control was given by ip to Swiss mice (6/sex/dose/time point, 15-20 g, 6-8 weeks). The mice were sacrificed after 30, 48 or 72 h of the treatment. Bone marrow slides from the femurs were prepared, and a total of 1000 polychromatic erythrocytes (PCE) were scored for each animal. The number of micronucleated PCE and NCE were counted.

Results
A dose of 170 or 340 mg/kg bw diuron significantly increased the number of micronuclei after 30 h and 48 h, but not after 72 h as compared to solvent control. The data from both sexes were combined since no sex dependent changes could be seen at 48 h time period. Diuron was considered to show the potential in the formation of micronuclei in mouse bone marrow cells.

MNPCE frequency (n = 6/sex)

	30 h	48 h	72 h
0 (DMSO)	1.3 ± 0.6	1.6 ± 0.6	1.6 ± 0.6
Diuron 85 mg/kg bw	3.6 ± 0.6	1.3 ± 0.6	1.6 ± 0.6
170	5.0 ± 1.6*	5.6 ± 2.1	1.6 ± 0.6
340	7.0 ± 1.0*	7.0 ± 1.7*	3.0 ± 1.7
Cyclophosphamide 20 mg/kg bw	4.0 ± 0.2*	6.0 ± 1.6*	4.2 ± 0.9*

Volkner W (1988). Mouse germ-cell cytogenetic assay with diuron. Lab: CCR Cytotest Cell Research GmbH & Co, KG Nieder-Ramstadter Stra e 146, D-6100 Darmstadt FRG. Report No: 49243. GLP/QA: yes.

Methods
NMRI male mice (6/dose/time point, 12 mice at 5000 mg/kg bw of diuron for 24 or 48 h) received a single dose of diuron 0 (solvent, 2% cremophor), 500, 1670 or 5000 mg/kg bw (Batch No: 232114123, Purity: 98.4%) or doxorubicin-hydrochlorid 7.5 mg/kg bw (positive control). The mice were sacrificed at 6, 24 or 48 h for the group at 5000 mg/kg bw of diuron, and at 24 h only for other groups, and germ cells from the testes were isolated and fixed for analysis of metaphase cells.

Results
Apathy occurred in all rats at 5000 mg/kg bw, and 6/12 died for the 24 h interval and 5/12 died for the 48 h interval. No toxic effects were reported for other dose levels.

Under the experimental conditions, the test substance did not induce chromosome aberrations in spermatogonial cells of the mouse.

Volkner W (1987a). Chromosome aberration test in bone marrow cells of the Chinese hamster with diuron. Lab: CCR Cytotest Cell Research GmbH & Co, KG Nieder-Ramstadter Stra e 146, D-6100 Darmstadt FRG. Report No: 49241. GLP/QA: yes.

Methods
Chinese hamster (6/sex/dose/time point, 12 at 5000 mg/kg bw of diuron for 48 h) received a single dose of diuron 0 (solvent, 2% cremophor), 500, 1670 or 5000 mg/kg bw (Batch No: 232114123, Purity: 98.4%) or cyclophosphamide 40 mg/kg bw (positive control). The animals were sacrificed at 6, 24 or 48 h for the group at 5000 mg/kg bw of diuron, and at 24 h only for other groups, and slices of the bone marrow from the femora were prepared for analysis of metaphase cells and scored for structural chromosome aberration (gaps, breaks, fragments, deletions, exchanges and chromosomal disintegration).

Results
Signs including abdominal position, eyelid closure and padding movements occurred in all rats at 5000 mg/kg bw, and 4 males and 2 females died for the 48 h interval. No toxic effects were reported for other dose levels.

Under the experimental conditions, the test substance did not induce chromosome aberrations in bone marrow cells of the Chinese hamster.

Volkner W (1987b). Sister chromatid exchange assay in bone marrow cells of the Chinese hamster with diuron. Lab: CCR Cytotest Cell Research GmbH & Co, KG Nieder-Ramstadter Stra e 146, D-6100 Darmstadt FRG. Report No: 49242. GLP/QA: yes.

Methods
Chinese hamster (6/sex/dose) received a single dose of diuron 0 (solvent, 2% cremophor), 500, 1670 or 5000 mg/kg bw (Batch No: 232114123, Purity: 98.4%) or cyclophosphamide 40 mg/kg bw (positive control), and were sacrificed 24 h post dosing. Slices of the bone marrow from the femora were prepared for analysis of metaphase cells for sister chromatid exchanges (reciprocal interchanges of the two chromatid arms of a single chromosome).

Results
Signs including abdominal position, eyelid closure and padding movements occurred in all rats at 5000 mg/kg bw, but none of the animals dies. No toxic effects were reported for other dose levels.

Under the experimental conditions, the test substance did not induce sister chromatid exchanges in bone marrow cells of the Chinese hamster.

Herbold B (1983). Diuron micronucleus test on the mouse to evaluate for mutagenic effects. Lab: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld. Report No: 49238. GLP/QA: no.

Methods
Bor NMRi mice (5/sex/dose) received a single oral dose of diuron 0 (solvent, 0.5% cremophor), or 2500 mg/kg bw (Batch No: 232114080, Purity: 98.8%) or endoxan 43.5 mg/kg bw (positive control). The mice were sacrificed at 24, 48 or 72 h for the group at 2500 mg/kg bw of diuron, and at 24 h only for other groups, and slides of the bone marrow were prepared for analysis of normochromatic and polychromatic erythrocytes.

Results
Mice treated with diuron 2500 did not show any ill-effects and there were no treatment-induced deaths.

Under the experimental conditions, the test substance did not cause micronucleus effects.

Herbold B (1998). Diuron micronucleus test on the mouse to evaluate for mutagenic effects. Lab: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld. Report No: 49238. GLP/QA: yes.

Methods
Bor NMRI mice (5/sex/dose) received a single dose of diuron 0 (solvent, 0.5% aqueous cremophor), or 700 mg/kg bw (Batch No: 232455681, Purity: 98.1%) or cyclophosphamide 100 mg (positive control) by ip. The mice were sacrificed at 16, 24 or 48 h for the group at 700 mg/kg bw of diuron, and at 24 h only for other groups, and slides of the bone marrow from femur were prepared for analysis of normochromatic and polychromatic erythrocytes.

Results
Mice treated an ip dose of diuron 700 mg/kg bw/day showed apathy, roughened fur, staggering gait, sternal recumbency, spasm, twitching, difficulty in breathing and eyelid stuck together, but none died during the study.

Under the experimental conditions, the test substance did not show a clastogenic effect.

10. HUMAN STUDIES

10.1 Percutaneous Absorption

No data.

10.2 Phototoxicity study

No data.

10.3 Occupational Exposure

Xavier R & Pereira G (2001). Medical surveillance from manufacturing site of diuron. Bayer AG, Agrochemical Centre Monheim, PF-E/REG, Gebaude 6100, D-51368 Leverkusen & Griffin (Europe) SA, Minervatstraat 8, B-1930 Zaventem.

It is stated that medical physical examination for employees did not reveal any exposure-induced clinical problems during 22 years of production of diuron.

Scarisbrick DA & Martin JW (1981). Biochemical changes associated with chloracne in workers exposed to tetrachlorazobenzene and tetrachlorazoxybenzene. J Soc Occup Med 31: 158-163.

In 1976 and 1977, workers on two linked plants producing diuron and its precursor 3,4-dichloraniline (DCA) developed mild chloracne. One year later review of the results of serial liver function tests together with lipid profiles in some of the workers revealed a higher than expected incidence of abnormalities. There were no significant abnormalities of liver function in the DCA/Diuron workers. However, there was a statistically significant increase in their mean triglyceride values (2.2 vs 1.4 mmol/L in control) which was greater in those who had chloracne. Mean cholesterol levels (5.51 vs 5.07 mmol/L in control) were also higher. As a result of a combined programme of improved occupational hygiene and personal health care the lipid values returned to normal.

A letter from Dr March Mil JE (Medical practitioner for annual occupational medical examinations) to Reuver, PF-A Beratung, Bayer: Diuron – evidence of chloracne. 1992.

Diuron with its potential impurities of TCAB and TCAOB did not cause any cases of chloracne in workers.

No cases of chloracne from areas of production of diuron have been known within the course of the company practice. The concentration of TCAB and TCAOB is reported to be very low and lies within the threshold of analytical determination.

10.4 Poisoning Incidents

Torrington KG (1983). Letters to the Editor. J Occupational Medicine 25: 354-356.

During the spring of 1982, two healthy young men, an engineer and conductor received an intense spray exposure to 2,4-D (2,4-dichlorophenoxyacetic acid) or Karmex. On the second day, both noted symptoms of itching and burning involving their oral and nasal mucosa and their conjunctiva. Small ulcerations appeared in areas where their skin had sweated or had made contact with glasses frames contaminated with the chemical. Within 24 hours, both men developed significant chest discomfort with cough, which was initially productive of a mucoid sputum. They complained of mild headache, some muscle twitching and throat soreness. Within the next 4-5 days, the cough and sputum production decreased, but both patients continued to feel somewhat weak and dyspneic. Initial complete blood counts were normal as were chest X-rays. Subsequent pulmonary function tests revealed no abnormalities of air flow, lung volumes or diffusing capacity. Both patients were concerned about the possibility of long-term pulmonary toxicity.

Geldmacher M, Mallinckrodt V & Schussler F (1971). Zu Stoffwechsel und toxizitat von 1-(3,4-dichlorphenyl)-3,3-dimethylharnstoff (diuron) beim menschen. Institut fur Rechtsmedizin der Universitat Erlangen-Nurnberg. Arch Toxikol 27:187-192. (Germ, English abstract).

After accidental ingestion of 38 mg/kg of diuron concomitantly with 20 mg/kg aminotriazole, a 39 year old woman showed no signs of intoxication. From a urine specimen taken some hours later, 1-(3,4-dichlorophenyl)-3-methylurea and 1-(3,4-dichlorophenyl)-urea could be isolated and identified. The urine specimen probably also contained some 3,4-dichloroaniline, but no unaltered diuron was demonstrated.

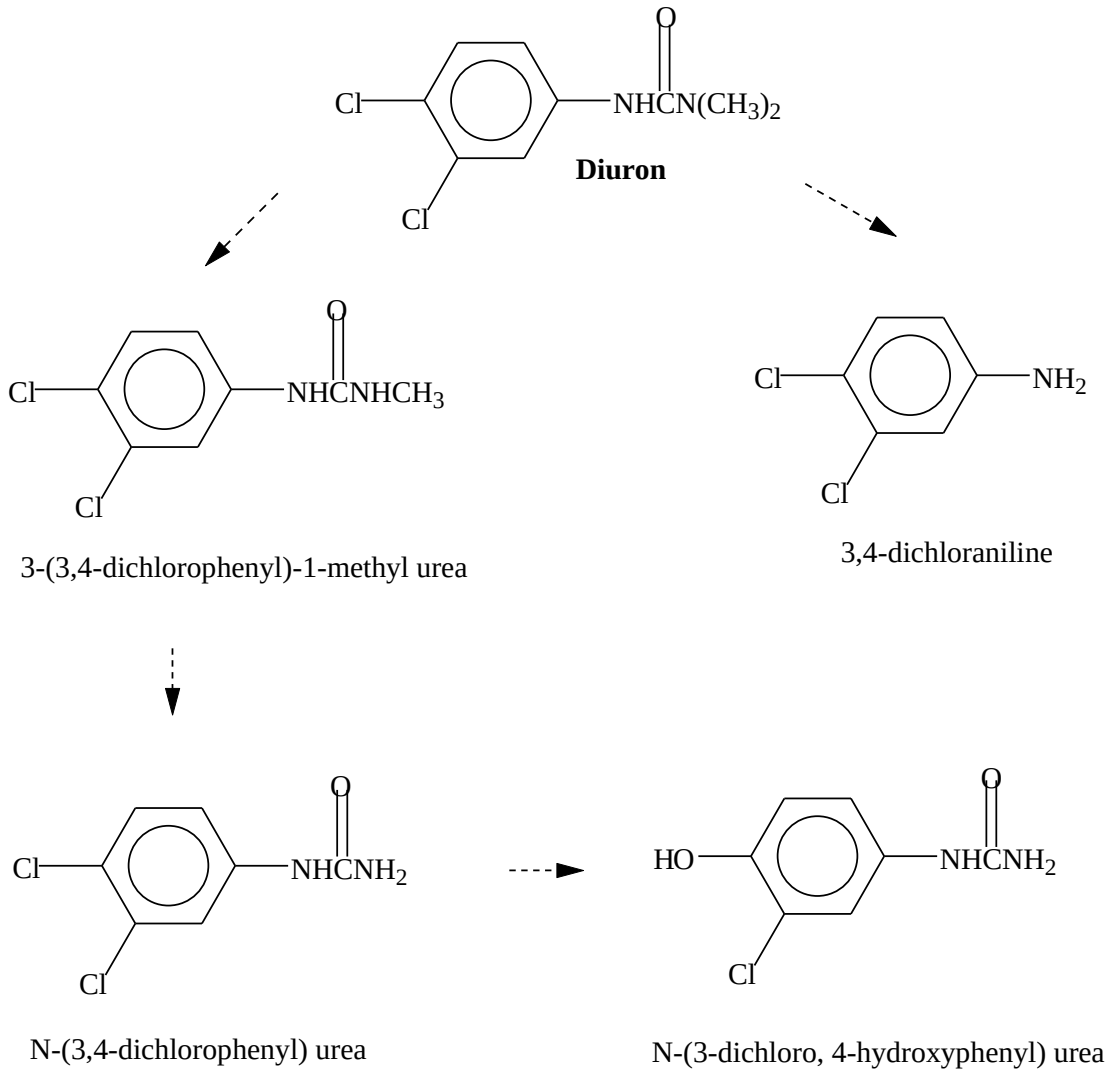
Verheij ER et al (1989). Identification of diuron and four of its metabolites in human post-mortem plasma and urine by LC/MS with a moving-belt interface. J Anal Toxi 13: 8-12.

Unknown compounds that were not amenable to GC/MS were found during routine benzodiazepine HPLC screening in a post-mortem case. The herbicide diuron and four of these metabolites were identified in plasma and urine and had a total concentration as high as 100 mg/L. Metabolism via demethylation and hydroxylation appeared to be the major routes.

Boven MV, Laruelle L & Daenens P (1990). HPLC analysis of diuron and metabolites in blood and urine. J Analytical Toxicology 14: 231-234. [Bayer CropScience Pty Ltd; Sub:CR101-1, Vol 25 of 40]

A 38-year old woman was brought into the emergency unit in a comatose state due to intoxication of the cholinesterase inhibitor parathion. The patient also ingested 38 mg/kg diuron. Although the patient did not develop particular symptoms relevant to diuron, diuron and its metabolites were found in the blood and urine of the patient by HPLC. Diuron metabolic pathways in humans were suggested as the following, which is similar to those in rats (see above studies, Wu D, 1996; and Pauluhn J & Eben D-C A, 1986).

Proposed Metabolic Pathways of Diuron in Humans



11. OTHER STUDIES

Immunotoxicity study

Vos JG, Krajnc EI, Beekhof PK and van Logten MJ (1983). Methods for testing immune effects of toxic chemicals: Evaluation of the immunotoxicity of various pesticides in the rats. In Pestic Chem: Human Welfare Environment, Proc. Int. Congr. Pestic Chem., 5th, Vol 3, 1983, 497-504.

Possible immunotoxic properties of a group of pesticides including diuron (oral doses of 25, 250 and 2500 mg/kg bw/day) were initially tested by a 3-week dietary study in weaned male rats, followed by a series of immune function tests in rats after weaning as well as pre- and postnatally. Diuron at 250 mg/kg bw/day and above increased the spleen weight. However, since large red pulp with increased extramedullar haemopoiesis and haemosiderin pigment deposits were also noticed, diuron was considered to have no or marginal effects on the immune system. No further tests were performed on diuron.

Mechanism Studies

Mice

Eiben R (1988). Chronic toxicity study on NMRI mice – Supplemental study to investigate haemotoxic effects (Administration in the feed for 6 months). Lab: Bayer AG, Institute for Toxicology, Wupprtal, Friedrich-Eblert-Strasse 217-233, West Germany. Report No 17373. Bayer Ag, D 51368 Leverkusen – Bayerwerk. Study Duration: December 1986 – June 1987. GLP/QA: yes.

Methods
The aim of this study was to investigate the haemotological effects observed at lower dosages in a carcinogenic study on NMRI mice, in particular during the first 6 months of the study. NMRI mice (30/sex/dose) received diuron at 0, 5, 25 or 250 ppm (0/0, 1.6/2.0, 7.5/10.1, 78/100 mg/kg bw/day for M/F by diet analysis) in the daily diet for up to 6 months. Mice were supplied by SPF-bred by Winkelmann, Borchen, and were 4-6 weeks old, 23-34 g for males and 22-27 g for females at the beginning of dosing. The dose selection was determined based on the 2-year carcinogenic study in mice (Report No: 19715). The mixture of diet (Altromin 1321 meal, by Altromin GmbH, Lage, ad libitum) with the test substance (Batch No: 232114080, Purity: 98.5-98.8%) was prepared weekly and the concentrations, homogeneity and stability of the test substance in the diet were analysed.

Clinical observation was made daily, detailed individual inspections, food consumption and body weight weekly. Blood samples were collected from non-fasted 10 mice/sex/dose under anaesthesia by ether on Weeks 5, 14 or 27, for examinations of haematology (Appendix V excluding clotting parameters and blood smear) and plasma bilirubin levels. Scheduled sacrifices were performed by exsanguination under anaesthesia with diethyl ether, selected organs (liver, spleen and kidneys) were weighed, and organs/tissues (eyes, Harder's gland, skin, bladder, liver, lung, kidneys, spleen, pancreas, spinal cord including cervical, thoracic and lumbar, skeletal muscle, sternum, femur, lymph nodes mandib and organs with gross abnormalities) were histo-pathologically examined.

Results
Two mice each at 5 and 250 ppm died following blood sample collection. Food consumption was not affected. Lower body weights (up to 10% lower) were observed in all treated groups, lacking any clear dose-relationship. Significantly higher levels of plasma bilirubin were observed at 25 and 250 ppm (2.9, 4.0 *vs* 2.3 in control, $p < 0.01$) at Week 4, but not at other test points.

Following dosing for up to 6 months, there were no treatment-related changes in haematology parameters, and no evidence of alteration in the haemopoietic system, i.e. neither increased medullary/extramedullary haemopoietic activity, nor increased haemosiderin deposition in the spleen or liver, was found.

Pathological examinations at different time intervals showed focal or single cell necroses, eosinophilic or granulocyte foci, round cell infiltrates in the liver of some mice from each group but without any dose-relationship. Increased siderosis or thrombopoiesis was observed in the spleen of single mice from treated groups as well as the control. Round cell infiltrates and basophilic tubuli were seen in the kidneys of all groups, more frequently in elderly mice, suggesting an age-related change. No other abnormal findings were revealed in the liver, kidneys, spleen and spinal cord at the selected dosages.

Rats

Sherman H (1962). Ninety-day feeding study with 3-(3,4-dichlorophenyl)-1,1-dimethyl dimethyl urea (diuron). Griffin L.L.C., 2509 Rocky Ford Road, Valdosta, GA 31601, USA. Report No: 51533 (97-62). Griffin Cooperation, P.O. Box 1847, and Rocky Ford Road, Valdosta, GA 31603-1847, USA.GLP: no.

Methods
The purpose of the study was to investigate some unexpected results (high mortality, depression of body weight and RBC counts at 250 and 2500 ppm, i.e. 0.025% and 0.25%), obtained in a 2-year rats feeding study (MRO-548, Report No: 51486, by Dr HC Hodge in the University of Rochester).

Charles River rats at weaning (20/sex/dose) received diuron as a 80% formulation at 0, 250 or 2500 ppm of the active ingredient in the diet for 90 days. The test substance (Code T 71113-D, Haskell No 3118, the same material used at the study MRO-548) was supplied by the Research Division of the Industrial and Biochemicals Department. The rats (CD albino) were average 82/76 g for M/F, and were

feed with Ground Purina Laboratory Chow plus 1% corn oil. Animals were weighed and food consumption was measured 2-3 times a week. Haematological examinations (RBC and WBC counts, haematocrit and haemoglobin) were conducted on 10 rats/sex/dose around Days 30, 60 and 90. At necropsy on Day 90, quantitative bone marrow counts were conducted on 10 rats per sex of the control and 2500 ppm groups. No clinical chemistry, urinalyses and pathological examinations were performed. No original data were present in the report.

Results
All animals but one survived to the study termination. One male at 250 ppm was found dead on day 53, with swollen salivary glands and congested and oedematous lungs. The cause of death was not clearly identified. Both male and female groups at 2500 ppm showed significantly reduced food consumption (up to 18-19%) and lower body weight (12-14% lower) throughout the entire test period.

Males and females at 2500 ppm exhibited lower RBC, haematocrits and haemoglobin values during the study. The average cell population of femoral marrow of this group was elevated (with or without correction for body weight) and more significant in females, suggesting an compensatory bone marrow hyperplasia. Higher values of WBC counts in male and female rats at 2500 ppm were at least partially due to the initial high counts of this group.

Changes in haematology on day 90 (n = 10/sex)

	male			**female**		
Dose (ppm)	**0**	**250**	**2500**	**0**	**250**	**2500**
RBC, 10^6/□L	7.94	7.80	6.71	7.53	6.51	6.07
Hct, %	46.5	46.1	44.4	45.1	42.2	40.9
Hb, g/L	160	154	144	159	142	134
WBC, 10^3/□□L	16.3	13.9	18.9	10.3	12.3	16.2

No statistic analysis was shown in the summary data.

In summary, the treatment at 2500 ppm, but not at 250 ppm, caused depression in food consumption, body weight gain and haematology. The high mortality observed in the 2-year dietary study (MRO-548) was not found in this study and is therefore considered to be unrelated to treatment.

Schmidt WM & Karbe E (1986a). Diuron: Toxicological study with Wistar rats paying special attention to effects on the blood (Administration in Diet for six months). Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, Western Germany. Report No: 14886 / Du Pont Report No: D/Tox 18. Study Duration: November 1984 – May 1985. GLP/QA: yes. Guideline: None.

Methods
In a chronic dietary rat study at 0, 25, 250 and 2500 ppm of diuron (Schmidt WM, Institute of Toxicology, Report No: 51470, Bayer AG. Report No 13962 of 29/10/1985), haematological changes in various erythrocyte parameters and morphological changes in blood-forming/regenerating tissues were noted at all dose levels. In order to establish a NOEL for diuron in relation to its effects on the erythrocytes, the following doses were selected in this study.

Wistar rats (10/sex/dose) received diuron at 0, 4, 10 or 25 ppm (0/0, 0.3/0.3, 0.7/0.8 or 1.6/1.8 mg/kg bw/day for M/F, Batch No: 232114123, Purity: 98.8%) in the diet for up to 26 weeks. Rats were received from Breeder Winkelmann, Borchen, and were 5 to 6 weeks old and 88-110 g for males and 85-110 g for females at start of the study. Food and test substance intake was determined weekly. Clinical observation was made daily, and a detailed examination and body weight measurement weekly. Blood samples were taken under ether anaesthesia on week 4, 12 and 26 week for haematological examinations (including RBC morphology). At termination on week 26, survivors were anaesthetised with diethyl ether before exsanguination. Liver and spleen weights were determined, organs/tissues from all animals were grossly examined, and selected organs (spleen, gross abnormalities in particular female urinary bladder) were histopathologically examined.

Results

One male and 1 female at 10 ppm died after the blood sampling, and the deaths were assumed to be anaesthetic over-doses or hypovolaemia. There were no treatment-induced clinical signs and changes in food consumption and body weight.

At 25 ppm, a slightly lower haemoglobin concentration in females and higher reticulocyte counts in both sexes were observed at various times. The histopathological examination and the morphometric evaluation revealed slightly increased accumulation of ferriferous pigments in the spleens of this group (presented as an increased degree and area of pigment accumulation), further indicating damage on the erythrocyte production.

Changes in haematology (n = 10/sex)

	male				female			
Dose (ppm)	**0**	**4**	**10**	**25**	**0**	**4**	**10**	**25**
Hb, g/L Wk 12	15.9	15.7	15.3*	15.5	15.0	14.8	14.8	14.3**
Wk 26	160	166	163	161	158	156	156	149*
Reti, %o Wk 12	10	11	16	19**	17	20	22	28*
Wk 26	14	13	10	12	12	15	15	18*

*P < 0.05; ** P< 0.01 by Mann-Whitney *U-test*.

At termination, absolute and relative weights of liver and spleen were comparable across all groups. Some treated animals exhibited dilation of blood vessel in the urinary bladder walls, increased consistency before filling with the fixative and/or reduced transparency after filling with fixative. However, automatic measurements of the bladder wall thickness did not prove a dose correlation.

The NOEL was 10 ppm (0.7/0.8 mg/kg bw/day for M/F) in this study based on reduced haemoglobin and increased reticulocytes at 25 ppm.

Schmidt WM & Karbe E (1986b). Study for toxicity to Wistar rats with special attention to urothelial alterations (Administration in Diet for 2, 4, 12 and 26 weeks with recovery). Lab: Institute of Toxicology, Bayer AG, Wuppertal, Friedrich-Ebert-Strasse 217-333, Western Germany. Report No: 15939. Study Duration: November 1984 – July 1985. GLP/QA: yes. Guideline: None.

Methods

In a chronic dietary rat study (Schmidt WM, Institute of Toxicology, Bayer AG. Report No: 13962 of 29/10/1985), tumours of the urothelium were noted at diuron 2500 ppm. The present study aimed to gain knowledge in morphogenesis of the tumours in Wistar rats, the time of origin and the reversibility of the cell proliferation. The scope of the study was limited to general observations (clinical signs, body weights, gross findings) and histopathology of the urinary bladder and kidneys.

Male Wistar rats (10/dose) received 0 or 2500 ppm of diuron in the daily diet for 2, 4, 12 or 26 weeks, or for 4 or 26 weeks followed by a recovery period of 4 or 8 weeks respectively. Rats were supplied by Breeder Winkelmann, Borchen, were 5 to 6 weeks old, and had a mean starting weight of 79 g. The mixtures of diet (Altromin 1321) with the test substance (Batch No: 232114123, Purity: 98.8%) were prepared weekly, and proved to be stable and homogeneous by diet analyses. Clinical observations were made daily, and detailed examinations and body weight measurement weekly. Rats were anaesthetised with diethylether, and sacrificed by exsanguination at scheduled times, and grossly examined. The thickness of the urinary bladder wall from all rats was measured, and histopathology of urinary bladders from all rats and kidneys from rats at 26-week dosing group only were examined.

Results

No rats died and no notable clinical signs were observed during the study. The treated rats gained less body weight (10-14% lower) during treatment, and the lower body weight (10% lower) was still apparent after the period of recovery (26 + 8 weeks).

Swelling or enlargement of the spleen with red-black discolouration was observed in 7/10 rats after 2-week dosing with 2500 ppm of diuron, and in all rats after a 4-week or longer dosing period. Similar alterations were not seen in the 4 + 4-week recovery group, and were in only 2/10 rats of the 26 + 8-week recovery group, indicating its reversibility.

Urinary bladders from treated rats showed increased consistency, reduced transparency and less dilated blood vessels than those from control rats, to an increasing extent as the duration of treatment increased. After 4-week dosing or longer, almost all rats exhibited hyperplasia of bladder epithelium that was associated with connective tissue proliferation and granulocyte infiltration, while the alterations were less frequent and severe in recovery groups. Hyperplasia with exo- and endophytic growth (within 4 weeks) and marked squamous epithelial metaplasia (after 26 weeks) were found. Distinct thickening of the urinary bladder wall in treated rats was indicated by increased areas of equally long wall regions from paramedian sections, and was largely the result of an increase in connective tissue, mainly in the subepithelial area. Recovery groups showed more or less a trend to reversion on cessation of treatment. Both the severity and the volume of hyperplasia were lower overall, and the thickening of the urinary bladder wall was no longer apparent or distinctly less remarked, in animals with 4- or 8-week recovery, compared with corresponding 4- or 26-week treatment only groups.

Number of rats with hyperplasia of urinary bladder epithelium (n=10/sex)

Dose (ppm)	**2 weeks**	**4 weeks**	**4 + 4-week recovery**	**12 weeks**	**26 weeks**	**26 + 8- week recovery**
0		1 fH1	1 fH1; 1 H1; 1 H2	1 fH1; 1 H1	2 fH1	1 fH1
2500	3 fH1	6 fH1; 1 H1; 1 H2; 1 H3; 1 H4	5 fH1; 2 H2	1 H1; 5 H2; 1 H3; 3 H4	1 fH1; 2 H1; 3 H2; 3 H5*	1 fH1; 2 H1; 1 H2; 1 H4; 2 H5

fH1: focal, simple hyperplasia; H1: simple hyperplasia with or without differentiation loss; H2: with epithelial neovascularisation and thicker than H1; H3: with epiphytic growth; H4: with endophytic growth; H5: with squamous epithelial metaplasia. * No bladder epithelium in section specimen from 1 rat.

After 26-week dosing, all rats exhibited slight epithelial hyperplasia (degree 1-2) in the renal pelvis, compared to 2/10 in control.

In conclusion, 2 weeks or longer treatment of rats with diuron 2500 ppm induced an increase in hyperplasia of urinary bladder epithelium, which was partially reversed by cessation of treatment. Some high degree hyperplasia may be regarded as a preliminary stage of the carcinomas found in the 2-year rat feeding study.

Pharmacology Studies

Algate DR et al (1989). General pharmacological evaluation of DCMU. Lab: Huntingdon Research Centre Ltd, PO Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Report No: HDY 254-261. GLP/QA: yes.

The general pharmacology of diuron (Batch No: 169, Purity: 98.9%) was evaluated using a battery of tests.

Effects on the CNS In the dose-range of diuron 30 – 1000 mg/kg bw, oral administration to mice induced no behavioural changes or signs of toxicity, except for a minor pilo-erection response which occurred in some mice receiving 100 mg/kg bw and above. Diuron at 100, 300 and 1000 mg/kg bw had no effect on hexobarbital-induced sleeping times in mice.

Effects on the respiratory and circulatory systems In 3 anaesthetised dogs, intraduodenal administration of vehicle (0.5% CMC) or diuron at 100, 300 and 1000 mg/kg bw failed to exert any noteworthy effects on blood pressure, heart rate, blood flow in a femoral artery, femoral resistance, and respiratory tidal volume, rate and minute volume. The minor fluctuations and trends in the basal

values of some parameters occasionally observed were not significantly modulated by the vehicle of test material in any animal at any dose.

Effects on the autonomic nerves system In 3 anaesthetised cats, intraduodenal administration of diuron at 100, 300 and 1000 mg/kg bw caused no overt changes in blood pressure and heart rate, and no consistent changes in the responses to bilateralcarotid occlusion of to noradrenaline. It similarly failed to modify the responses of the nictitating membrane to stimulation of the pre- and post-ganglionic nerves.

Effects on the gastro-intestinal tract Oral doses of diuron at 100, 300 and 1000 mg/kg bw produced a significant inhibition of intestinal motility at 1000 mg/kg bw in mice.

Effects on skeletal muscle Oral administration of diuron at 30, 100, 300 and 1000 mg/kg bw caused no significant changes in performance time on the rotarod in trained mice in a accelerating rotarod test.

Effects on bloodOral administration of diuron at doses up to 2000 mg/kg bw did not alter the prothrombin time or activated partial thromboplastin time of rat blood taken from the retro-orbital sinus and by tail stab respectively. Diuron at 600 and 2000 mg/kg bw caused an increase in the activated partial thromboplastin time, but the value for the majority of animals remained within the normal range. In an *in vitro* haemolytic test on human erythrocytes, diuron at 0.03, 0.1, 0.3 and 1.0 mg/mL exerted virtually no haemolytic effect when mixed with a 3% suspension of human erythrocytes.

Impurity studies

3,4,3',4'-tetrachloroazobenzene [lot No. M-989]

	Species [strain]	**Sex**	**Group Size**	**Vehicle**	**Doses Tested (mg/kg bw or mg/m^3)**	**LD50 (mg/kg bw or mg/m^3)**	**Reference**
Oral	Rat (ChR-CD)	M	10/D	Corn oil	5 000	> 5 000 (no death)	Kaplan (1975)
Dermal	Rabbit [albino]	M	1/dose	DMSO	0, 130 – 1000 (24 h)	No death at 1000	Morrow (1976)
Inhalation	Rat (ChR-CD)	M	6/D	Generating air by heating	590, 880, 920, 970	880 - 920 (estimation)	Trochimowicz (1976)

Abbreviations: NZW=New Zealand White; DMSO: dimethyl sulfoxide

Morrow (1976). Skin acnegenesis (Dow method).

To establish acceptable levels for clean-up of the contaminated operating building, "Incident tars" (3,4-dichloroaniline, at 10, 1, 0.1 and 0.01%) and TCAB (3,3',4,4'-tetrachloroazobenzene, at 2, 0.2, 0.02 and 0.002%) were compared by applying 2 drops of each solution to the distal half of the inner aspect of the left ear of a rabbit for 5 days/week over a 4 week period, or 6 weeks for the lowest concentration of each. Dose-related responses from slight to severe sloughing, plug formation, ear thickening and hair lose were observed for both test materials, with the highest concentration of TCAB (2%) was more severe than that of Incident tars (10%). Both are strong acnegens.

Trochimowicz (1976). Two-week inhalation toxicity study on TCAB.

A group of 6 male Chr-CD rats were exposed to volatilised 3,3',4,4'-tetrachloroazobenzene (TCAB) at 190 mg/m3, or an air control, for 4 hours a day, 5 days a week over 2 weeks. Three rats were killed for pathology examination after the 2-week exposure, and the rest were observed for 14 days post-exposure. No clinical signs or pathologic changes were observed. After the 10th exposure, increased RBC counts (~12%) as well as decreased mean cell volume (~10-15%) and mean cell haemoglobin (~10-15%) were detected in the test group after the 10th exposure. Haematological changes were similar in trend after 14-day observation. Some rats had darker urine with higher pH and sugar positive.

APPENDIX I: DIURON TOXICOLOGY DATA SUBMISSION DETAILS

Confidential Business Information - removed

APPENDIX II: ACTIVE CONSTITUENT APPROVAL HOLDERS

(refer to summary document for up to date list)

APPENDIX III: COMPOSITION OF ACTIVE DIURON

Confidential Business Information - removed

APPENDIX IV: AUSTRALIAN REGISTERED PRODUCTS CONTAINING DIURON

(refer to PUBCRIS for up to date list)

APPENDIX V: LIST OF CLINICAL CHEMISTRY, HAEMATOLOGY & URINALYSIS PARAMETERS

Clinical Chemistry	**Haematology**	**Urinalyses**
Albumin alkaline phosphatase bilirubin (total) calcium chloride cholesterol (total) cholinesterase activity creatinine (blood) gamma-glutamyl transpeptidase globulin glucose (blood) LDH (serum lactate dehydrogenase) Phosphorus potassium protein (total) SGPT (serum alanine aminotransferase) SGOT (serum aspartate aminotransferase) Sodium triglycerides urea nitrogen (blood) CPK (creatinine phosphokinase)	clotting parameters (clotting time, prothrombin time) erythrocyte count haematocrit (packed cell volume) haemoglobin leucocyte differential count leucocyte total count platelet count reticulocyte count MCH MCHC MCV blood smear	appearance specific gravity glucose ketones sediment (microscopic) occult blood pH protein volume bilirubin urobilinogen

APPENDIX VI: ORGANS FOR WEIGHT DETERMINATION AND HISTOPATHOLOGICAL EXAMINATION

Organs Weighed	Tissues Examined		
Adrenals	Adrenals	heart	prostate
Brain	aorta	ileum	rectum
Gonads	blood smear	jejunum	salivary gland
Heart	bone	kidneys	seminal vesicle
Kidneys	bone marrow	lacrimal gland	skin
Liver	brain (3 levels)	liver	spinal cord (cervical
Spleen	cecum	lungs	thoracic, lumbar)

Organs Weighed	Tissues Examined		
Thyroid (w/parathyroid)	colon duodenum epididymes eyes eyes (optic nerve) gall bladder Harderian glands head - 3 sections (nasal cavity, para-nasal sinus, tongue, oral cavity, naso-pharynx, inner-ear)	lymph nodes mammary gland muscle (smooth) muscle (skeletal) nerve (peripheral) oesophagus ovaries pancreas pituitary	spleen sternum stomach testes thymus thyroid (w/parathyroid) trachea urinary bladder uterus vagina Zymbal's gland gross lesions

APPENDIX VII: REPRODUCTIVE AND DEVELOPMENTAL INDICES

$$\textbf{Male/female mating index } (\%) = \frac{\text{number of males/females with confirmed mating*}}{\text{number of males/females placed with females/males}} \times 100$$

* defined by females with vaginal sperm or that gave birth to a litter or with pups/fetuses in utero

$$\textbf{Male fertility index (\%)} = \frac{\text{number of males proving their fertility*}}{\text{number of males placed with females/males}} \times 100$$

* defined by a female giving bith to a litter or with pups/fetuses in utero

$$\textbf{Female fertility index (\%)} = \frac{\text{number of females pregnant*}}{\text{number of females mated**}} \times 100$$

* defined as the number of females that gave birth to a litter or with pup/fetuses in utero

** defined as the number of females with vaginal sperm or that gave birth to a litter or with pups/fetuses in utero

$$\textbf{Gestation index (\%)} = \frac{\text{number of females with live pups on the day of birth}}{\text{number of females pregnant*}} \times 100$$

* defined as the number of females that gave birth to a litter or with pups/fetuses in utero

$$\textbf{Live birth index (\%)} = \frac{\text{number of liveborn pups at birth}}{\text{total number of pups born}} \times 100$$

$$\textbf{Viability index (\%)} = \frac{\text{number of live pups on day 4* after birth}}{\text{number of liveborn pups on the day of birth}} \times 100$$

* before standardisation of litters (i.e. before culling)

$$\textbf{Lactation index (\%)} = \frac{\text{number of live pups on day 21 after birth}}{\text{number of live pups on day 4* after birth}} .$$

* after standardisation of litters (i.e. after culling)

$$\textbf{Sex ratio} = \frac{\text{number of live male or female pups on day 0/21}}{\text{number of live male and female pups on day 0/21}} \times 100$$

$$\textbf{Conception rate (\%)} = \frac{\text{number of pregnant animals}}{\text{number of fertilised animals}} \times 100$$

$$\textbf{Preimplantation loss (\%)} = \frac{\text{number of corpora lutea – number of implantations}}{\text{number of corpora lutea}} \times 100$$

$$\textbf{Postimplantation loss (\%)} = \frac{\text{number of implantations – number of live foetuses}}{\text{number of implantation}} \times 100$$

APPENDIX VIII - REFERENCES

TOXICOLOGY

Agrawal RC, Kumar S & Mehrotra NK (1996). Micronucleus induction by diuron in mouse bone marrow. Toxicology Letters 89: 1-4.

Algate DR et al (1989). General pharmacological evaluation of DCMU. Lab: Huntingdon Research Centre Ltd, PO Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Report No: 51579.

Arce GT (1984). Mutagenicity Evaluation of diuron in Salmonella Typhimurium. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, 19714 USA. Report No: 52015.

Arce GT (1985). Assessment of diuron in the in vitro unscheduled DNA synthesis assay in primary rat hepatocytes. Lab: EI du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report No: 51553.

Arce GT (1987a). Mutagenicity Evaluation of diuron in Salmonella Typhimurium (Supplement 1). Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, 19714 USA. Report No: 51559

Arce GT (1987b). Mutagenicity Evaluation of diuron in Salmonella Typhimurium (revised). Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, 19714 USA. Report No: 51558.

Arce GT (2001). Diuron: cancer classification and mechanism of action. Grrifin LLC, 2509 Rocky Ford Road, Valdosta GA 31601. Report No: 52119.

Bakili RA (2000). Primary dermal irritation study of diuron technical 97% in rabbits. Lab: Department of Toxicology, JAI Research Foundation, Valvada – 396 108 Dist. Valsad, Gujarat India.

Bakili RA (2000). Primary eye irritation study of diuron technical 97% in rabbits. Lab: Department of Toxicology, JAI Research Foundation, Valvada – 396 108 Dist. Valsad, Gujarat India.

Bakili RA (1999). Dermal sensitisation study of diuron technical 97% in guinea pigs [Buehler test). Lab: Department of Toxicology, JAI Research Foundation, Valvada – 396 108 Dist. Valsad, Gujarat India.

Beres E (1986a). Acute inhalation toxicity test of diuron technical active ingredient on Wistar rat. NEVIKI Research Institute for Heavy Chemical Industries. Veszprem.

Beres E (1986b). Investigation of oral cumulative toxicity of diuron technical active substance on both sex Wistar rat. NEVIKI Research Institute for Heavy Chemical Industries. Veszprem.

Beres E (1986c). 90 days feeding study of diuron technical active ingredient on Wistar rat. NEVIKI Research Institute for Heavy Chemical Industries. Veszprem.

Boven MV, Laruelle L & Daenens P (1990). HPLC analysis of diuron and metabolites in blood and urine. J Analytical Toxicology 14: 231-234.

Boyd D & Krupa V (1970). Protein-deficient diet and diuron toxicity. J Agr Food Chem. 18:1104-7.

Cook, JC (1990). Reproductive and fertility effects with diuron (IN 14740): Multigeneration reproduction study in rats. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714, USA. Report no: 51525 & 51526.

Cox LR (1985). In vivo assay of diuron for chromosome aberrations in rat bone marrow cells. Lab: EI du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report No: 51554.

Cox LR (1997a). In vivo assay of diuron for chromosome aberrations in rat bone marrow cells (Revised report). Lab: EI du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report No: 51555.

Cox LR (1997b). In vivo assay of diuron for chromosome aberrations in rat bone marrow cells (Revision No 2). Lab: EI du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report No: 51556.

Dearlove GE (1986a) Developmental toxicity study of H-16035 administered by gavage to rats. Lab: Argus Research Laboratories, Inc, Horsham, Pennsylvania 19044 USA. Report No: 51545.

Dearlove GE (1987a) Developmental toxicity study of H-16035 administered by gavage to rats (Supplement 1). Lab: Argus Research Laboratories, Inc, Horsham, Pennsylvania 19044 USA. Report No: 51546.

Dearlove GE (1986b). Developmenal toxicity study of H-16035 administered by gavage to New Zealand White rabbits. Lab: Argus research laboratories, Inc., 935 Horsham Road, Horsham, Pennsylvania 19044 USA. Report No. 51547.

Dearlove GE (1987b). Developmenal toxicity study of H-16035 administered by gavage to New Zealand White rabbits (Supplement 1). Lab: Argus research laboratories, Inc., 935 Horsham Road, Horsham, Pennsylvania 19044 USA. Report No. 51548.

Dunsire JP, Johnston AM & Jack L (1994). The metabolism and distribution of [Phenyl(U)-14C] diuron in laying hens (Revision No 1). Lab: Inveresk Research International Tranent, EH33 2NE, Scotland. Sponser: EI DuPond de Nemours and Company, Agricultural Products Department, Experimental Station, Wilmington, Delaware 19880-0402. Report no: AMR 2171-91.

Eiben R (1988). Chronic toxicity study on NMRI mice-supplemental study to investigate haemotoxic effects (administration in the feed for 6 months – Part 1 of 3. Lab: Bayer AG, Institute for Toxicology, Wupprtal , Friedrich-Eblert-Strasse 217-233, West Germany. Report No: 17373.

Eiben R, Laliner G, Karbe E, Suberg H (1990). Diuron: Chronic toxicity and carcinogenicity with NMRI mice (administration in diet for 24 months) Volumes 1-3. Lab: Bayer AG, Institute for Toxicology, Wuppertal, Friedrich-Ebert-Strasse 271-233, West Germany. Report No: 51492, 51493 & 51494.

Eiben R, Laliner G, Karbe E, Suberg H (1994). Volume I of supplementary data supporting the diuron 2-year feeding study in NMRI mice. Lab: EI du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report No: 51519.

Faul J (1980). Diuron-evidence of chloracne. Bayer AG, Institute for Toxicology, Medical Department, Dormagen, Germany & Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany.

Faul J (1982). Statement to Pkt IV/1.2.2 of the BBA application form “Details of effects on man, internal company experience. Bayer AG, Institute for Toxicology, Medical Department, Dormagen, Germany & Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, Germany.

Geldmacher M, Mallinckrodt V&Schussler F (1971). Zu Stoffwechsel und toxizitat von 1-(3,4-dichlorphenyl)-3,3-dimethylharnstoff (diuron) beim menschen. Arch. Toxikol 27:187-192. Lab: Institut fur Rechtsmedizin der Universitat Erlangen-Nurnberg. Report no: not stated.

Heimann KG (1981). Diuron determination of acute toxicity (LD50). Lab: Bayer AG Institute for Toxicology, Wuppertal-Elberfeld, Germany. Report No: not stated.

Heimann KG (2001). Diuron: assessment of reversibility of urinary bladder effects after chronic oral application to rats. Lab: Bayer AG, Business Group Crop Protection, Development / Registration, D-51368 Leverkusen, Germany.

Heimann KG & Thyssen J (1983). Diuron active ingredient studies on acute toxicity. Lab: Bayer AG Institute for Toxicology, Wuppertal-Elberfeld, Germany. Report No: 11710.

Henry JE (1987). Dermal sensitisation study with IN-14,740-146 in guinea pigs. Lab: EI du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report No: 51444.

Herbold B (1984a). Diuron: Salmonella / microsome test to evaluate for potential point mutation. Lab: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Report No: 49237.

Herbold B (1984b). POL test on E Coli to Evaluate for potential DNA damage. Lab: Bayer AG Institute of Toxicology, Wuppertal-Elberfeld. Report No: 49239.

Herbold BA (1983). Micronucleus test on the mouse to evaluate for mutagenic effect. Lab: Bayer A, Indtitute of Toxicology, Wuppertal FRG. Report No: 49238.

Herbold BA (1998a). Diuron: Micronucleus test on the mouse. Lab: Bayer A, Indtitute of Toxicology, Wuppertal FRG. Report No: 49244.

Herbold BA (1998b). On the relevance of the data presented by Agrawal et al. in toxicological letters 89, 1996, 1-4. Entitiled “Microculeus induction by diuron in mouse bone marrow” (Internal memo). Bayer AG, PH-PD Toxicology, Carcinogenicity and Genotoxicity.

Herbold BA (1989). In vitro cytogenetic study with human lymphocytes for the detection of induced clastogenic effects. Lab: Bayer AG, Indtitute of Toxicology, Wuppertal FRG. Report No: 49246.

Herbold B (1985). Diuron: Dominant lethal test on the male mouse to evaluate for mutagenic effect. Lab: Bayer AG, Institute of Toxicology. Study No: 49245

Hodge HC (1964). Short-term and chronic feeding studies with diuron in rats and dogs – An interpretive summary. Department of Pharmacology, University of Rochester, School of Medicine and Dentistry. Report No: 51491.

Hodge H C & Downs W L (1964). Chronic feeding studies of diuron in rats. Department of Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA. Report No: 51486.

Hodge HC & Downs WL (1964). Chronic feeding studies of diuron in dogs. Department of Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA. Report No. 51485.

Hoffmann K & Schilde B (1985). Diuron-chronic toxicity to dogs after oral administration (12-month feeding study). Bayer Ag, Institute for Toxicology, Wuppertal-Elberfedl, Germany. Report No: 51521.

Joshi G (2000). Acute inhalation toxicity study of diuron technical 97% in rats. Lab: Department of Toxicology, JAI Research Foundation, Valvada – 396 108 Dist. Valsad, Gujarat India.

Kamath HG (1998). Genetic toxicology: Salmonella typhimurium reverse mutation assay with Sanachem Diuron Technical. Lab: Toxicology Department, Rallis Research Centre, Rallis India Limited, Peenya II Phase, Bangalore 560 058 India. Sponsor: Sanachem (Pty) Limited, Old Mill Site Canelanda 4341, Durban 400, South Africa. Report No: 48720.

Kalra SK & Chahal KS (1979). Degradation of diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea in cows. Ecotoxicology Environmental Safety 3: 362-368. Report No: 51568

Kaplan AM (1975). Oral LD50 test. Lot No: M-989. Lab: E I du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Lab Report No: 51374.

Kasa Z (1986). Study on the acute toxicity of diuron technical active ingredient Vol I. (Acute oral toxicity in rat, acute percutaneous toxicity in rat, acute intra-peritoneal toxicity in rat, acute eye irritant effect in rabbit, acute dermal irritant effect in rabbit). NEVIKI Research Institute for Heavy Chemical Industries. Veszprem.

Kasa Z (1986). Study on the acute toxicity of diuron technical active ingredient Vol II (Acute oral toxicity in mice). NEVIKI Research Institute for Heavy Chemical Industries.

Kasa Z (1986). Acute skin irritation and sensitisation testing of diuron technical active ingredient on guinea pigs. NEVIKI Research Institute for Heavy Chemical Industries. Veszprem.

Kinney LA (1987). Acute inhalation toxicity study with diuron in rats. Code: IN-14740-146. Lab/Sponsor: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware / Du Pont Agricultural Products, Wilmington, Delaware. Report No: 51468.

Kiss I (1986). Mutagenicity studies on diuron technical active substance. (Ames test, Micronucleus test, test of DNA damage by Sister Chromatid Exchange (SCE) test in CHO-KI cells. Lab: NEVIKI Research Institute for Heavy Chemical Industries. Veszprem.

Klein W (1986). Induction of and effect on UDS by test substance diuron. Lab: Austrian Research Center, Seibersdorf, Ges m b H A-2444, Seibersdorf. Report No: 49240.

Kurinnyi AI (1977). The mutagenicity of certain pesticides – derivatives of urea, carbamic and thiocarbamic acids. Cytology and Genetics 11: 62-64.

Madison WA (1983) Acute dermal toxicity – Method, summary, pathology. Primary dermal irritation – Method, summary. Primary eye irritation – Method, summary. Lot: 306202. Lab/Sponsor: Hazleton Raltech, Inc. Madison WI 53707. Report No: 11845.

Malek DE (1997). Volume 2 of Supplementary data supporting the diuron 2-year feeding study in rats. Lab: EI du Pont de Nemours and Compan, DuPont Agricultural Products, Barley Mill Plaza, Wilmington DE 19880-0038, USA. Report No: 51484.

Malek DE (1997). Cancer hazard characterization of diuron. EI du Pont de Nemours and Company, DuPont Agricultural Products, Barley Mill Plaza, Wilmington, Delaware 19880-0038 USA. Report No: 51520.

McKenzie SA (1992). Repeated dose dermal toxicity: 21-day study with DPX-14740-166 (diuron) in rabbits (revised No 1). Lab: Du Pont Agricultural Products, E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report No: 51541.

Mihail D-BF & Schilde B (1984). Diuron subacute dermal toxicity study on rabbits. Lab: Institute of Toxicology, Bayer AG, Woppertal-Elberfeld.. Report-no. 12360. Study No: T3016060. Study Duration: June – July 1983.

Morrow RW (1976). Acute skin absorption on rabbits – ALD. Lot No: M-989. Lab: E I du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51381.

Morrow RW (1976). Skin acnegenesis (Dow method). Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51437.

Needour C (1988). Response to CDFA's review of the diuron chronic rat study (Position paper). Report No: 51480.

Oellrich W (2001). Diuron – Summary of the toxicological and metabolism studies on the active substance. Lab: Bayer AG, Agrochemical Centre Monheim, D-51368 Leverkusen & Griffin (Europe) SA, Minervatstraat 8, B-1930 Zaventem.

Pauluhn J (1986). Diuron: Study for subacute inhalation toxicity to the rat (aerosol exposure 15 x 6 hours). Lab: Bayer AG, Institute for Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-233, West Germany.

Pauluhn J (1986b). Diuron: Study for subacute inhalation toxicity to the rat (aerosol exposure for 4 and 8 weeks). Lab: Bayer AG, Institute for Toxicology, Wuppertal-Elbertfelf. Report No: 14603.

Pauluhn J & Eben D-C A (1986). The concentration of diuron and its representative metabolites in the urine of male and female rats during a subacute inhalation study over eight weeks. Lab: Bayer AG, Fachbereich Toxikologie. Report No. 14754.

Rajwani LS (1999). Salmonella typhimurium reverse mutation assay of diuron technical. Lab: Microbiology Section, Department of Toxicology, JAI Research Foundation, Valvada – 396 108, Dist. Valsad Gujarat India. Sponsor: Ancom Crop Care Sdn. Bhd., 102 Block A, Phileo Damansara 1, Jalan 16/11, 46350 Petaling Jaya, Selangor Darul Ehsan, Malaysia.

Rana MD (1999). Acute dermal toxicity study of diuron technical 97% in rats. Lab: Department of Toxicology, JAI Research Foundation, Valvada – 396 108 Dist. Valsad, Gujarat India.

Rana MD (1999). Acute oral toxicity study of diuron technical 97% in rats. Lab: Department of Toxicology, JAI Research Foundation, Valvada – 396 108 Dist. Valsad, Gujarat India.

Reynolds VL (1992a). Mutagenicity testing of IN-15926-6 in the Salmonella Typhimurium plate assay. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, 19714 USA. Report No: 51561.

Reynolds VL (1992b). Mutagenicity testing of DPX-12,402-46 in the Salmonella typhimurium plate assay. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, 19714 USA. Report No: 51561.

Reynolds VL (1992c). Mutagenicity testing of IN-12894-5 in the Salmonella typhimurium plate incorporation assay. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, 19714 USA. Report No: 51563.

Rossberg WM (1995). Volume 1 of Supplementary data supporting the diuron 2-year feeding study in rats. Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Report No: 51481.

Rossberg WM & Wirnitzer U (1994). Diuron: Study for chronic toxicity and cancerogenicity with Wistar rats (Administration in diet for up to two years, Addendum to Bayer-report no. 13962). Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Report No: 6548.

Rossberg WM & Wirnitzer U (1994). Diuron: Study for chronic toxicity and cancerogenicity with Wistar rats (Administration in diet for up to two years, Second addendum to Bayer-report no. 13962 dated October 10, 1985). Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Report No: 6548.

Rossberg WM & Wirnitzer U (1995). Study for chronic toxicity and cancerogenicity with Wistar rats (Addendum 1). Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Report No: 51482.

Rossberg WM & Eiben R (1997). Diuron: Study for chronic toxicity and cancerogenity with Wistar rats (Administration in diet for up to two years – Second amendment). Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Report No: 51483.

Sarver JW (1992). Approximate lethal dose (ALD) of IN-12894-5 in rats. Medical Research No: 4581-951. Lab/Sponsor: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware / Du Pont Agricultural Products, Wilmington, Delaware. Report No: 51375.

Sarver JW (1992). Approximate lethal dose (ALD) of IN-12894-6 in rats. Medical Research No: 4581-952. Lab/Sponsor: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware / Du Pont Agricultural Products, Wilmington, Delaware. Report No: 51376.

Scarisbrick DA & Martin JW (1981). Biochemical changes associated with chloracne in workers exposed to tetrachlorazobenzene and tetrachlorazoxybenzene. J Soc Occup Med 31: 158-163.

Schmidt WM (1985). Diuron: Study for chronic toxicity and carcinogenicity with Wistar rats (Administration in diet for up to two years). Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Report No: 51470 & 51471.

Schmidt WM & Karbe E (1986). Diuron: Toxicological study with Wistar rats paying special attention to effects on the blood (Administration in Diet for six months). Lab: Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Dbert-Strasse 217-333, Western Germany. Report No: 51536 & 51540.

Schmidt WM & Karbe E (1986b). Study for toxicity to Wistar rats with special attention to urothelial alterations (Administration in Diet for 2, 4, 12 and 26 weeks with recovery). Lab: Institute of Toxicology, Bayer AG, Wuppertal, Friedrich-Dbert-Strasse 217-333, Western Germany. Report No: 51524.

Schneider PW (1984). “Karmex” toxicity summary. Report No: 51574.

Seiler JP (1978). Herbicidal phenylalkylureas as possible mutagens. I. Mutagenicity tests with some urea herbicides. Mutation Research 58: 353-359.

Sharp VW (1997a). Acute eye irritation/corrosion test with Sanachem diuron technical in rabbits. Lab: Biocon Research (Pty) Ltd, Pretoria 0001, South Africa. GLP/QA: yes. Guideline: OECD 405. Report No: 48714.

Sharp VW (1997b) Acute dermal irritation/corrosion test with Sanachem diuron technical in rabbits. Lab: Biocon Research (Pty) Ltd, Pretoria 0001, South Africa. Report No: 48713.

Sharp VW (1997c). Contact hypersensitivity to Sanachem Diuron Technical in albino guinea pigs (Magnusson Kligman Maximization Test). Lab: Biocon Research (Pty) Ltd, Pretoria 0001, South Africa. Report No: 48712.

Sherman H (1962). Ninety-day feeding study with 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (diuron). Griffin L.L.C., 2509 Rocky Ford Road, Valdosta, GA 31601, USA. Medical Research Project Nos: MR-631 & MR-10. Report-no.97-62. Griffin Cooperation, P.O. Box 1847, and Rocky Ford Road, Valdosta, GA 31603-1847, USA. Report No: 51533.

Shivaram S (1998). Genetic toxicology: in vitro mammalian cell gene mutation test with Smachem Diuron Technical. Lab: Toxicology Department, Rallis Research Centre, Rallis India Limited, Peenya II Phase, Bangalore 560 058 India. Sponsor: Sanachem (Pty) Limited, Old Mill Site Canelanda 4341, Durban 400, South Africa. GLP/QA: yes. Report No: 48719.

Shivaram S (1999). Genetic toxicology: in vitro mammalian cytogenetic test with Smachem Diuron Technical. Lab: Toxicology Department, Rallis Research Centre, Rallis India Limited, Peenya II

Phase, Bangalore 560 058 India. Sponsor: Sanachem (Pty) Limited, Old Mill Site Canelanda 4341, Durban 400, South Africa. Report No: 48721.

Torrington KG (1983). Letters to the Editor. J Occupational Medicine 25: 354-356.

Trochimowicz (1976). Two-week inhalation toxicity study on TCAB. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51438.

Trochimowicz HJ (1976). Approximate lethal concentration (ALC). Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51467.

Tronnier H (1983). Diuron: Expertise by consultant dermatologist on testing for phototoxicity in test subjects. Report No: R 2543.

Ullman DV & Choy WN (1985). Mutagenicity evaluation of diuron in the CHO/HPRT assay. Lab: EI du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report No: 51557.

Ullman DV (1985). In vivo assay of diuron for chromosome aberrations in rat bone marrow cells. Lab: EI du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report No: 51554.

Van Pelt CS & Hoffman K (1993). Supplement to diuron chronic toxicity to dogs after oral administration (12-month feeding study). Lab: Bayer Ag, Institute for Toxicology, Wuppertal-Elberfeld, Germany. Report No: 51522.

Verheij ER et al (1989). Identification of diuron and four of its metabolites in human post-mortem plasma and urine by LC/MS with a moving-belt interface. J Anal Toxi 13: 8-12.

Volkner W (1987a). Chromosome aberration test in bone marrow cells of the Chinese hamster with diuron. Lab: CCR Cytotest Cell Research GmbH & Co. KG, Nieder-Ramstadter Stra□e 146. D-6100 Darmstadt GRG. Report No: 49241.

Volkner W (1987a). Sister chromatid exchange assay in bone marrow cells of the Chinese hamster with diuron. Lab: CCR Cytotest Cell Research GmbH & Co. KG, Nieder-Ramstadter Stra□e 146. D-6100 Darmstadt GRG. Report No: 49242.

Volkner W (1988). Mouse germ-cell cytogenetic assay with diuron. Lab: CCR Cytotest Cell Research GmbH & Co. KG, Nieder-Ramstadter Stra□e 146. D-6100 Darmstadt GRG. Report No: 49243.

Vos JG, Krajnc EI, Beekhof PK and van Logten MJ (1983). Methods for testing immune effects of toxic chemicals: Evaluation of the immunotoxicity of various pesticides in the rats. In Pestic Chem: Human Welfare Environment, Proc. Int. Congr. Pestic Chem., 5th, 3: 497-504.

Wandrag S (1993). Oral and Dermal limit test with Sanachem Sanuron (Diuron technical) in rats. Batch: 292-03-93. Report No: 1. Lab/Sponsor: Roodeplaat Research Laboratories (Pty) / Sanachem (Pty) Ltd. Ltd. Report No: 48715.

Wandrag S (1996a). Subchronic oral toxicity – rodent: 90-day study with Sanachem diuron technical in rats. Lab: Biocon Research (Pty) Ltd, Pretoria, South Africa. Sponsor: Sanachem (Pty) Ltd, Durban, South Africa. Report No: 48716.

Wandrag S (1996b). Subchronic dermal toxicity – rodent: 90-day study with Sanachem diuron technical in rats. Lab: Biocom Research (Pty) Ltd, Pretoria, South Africa. Sponsor: Sanachem (Pty) Ltd, Durban, South Africa. Report No: 48717.

Weber H & Abbink J (1988). [Phenyl-UL-14C]-diuron: Investigation of the biokinetic behaviour in the rat. Lab: Institute for Metabolism Research, Metabolism Animal, PF-Zentrum Monheim, Building 6670, 5090 Leverkusen-Bayerwerk. Report No: 51571.

Wu D (1996). Absorption, distribution, metabolism and elimination of [14C]-diuron in rats. Lab: XenoBiotic Laboratories, Inc. Plainsvoro NJ 08536 & EI du Pont de Nemours and Company Experimental Station, Wilmington DE 19880-0402. DuPont Report No: AMR 3145-94.

Xavier R & Pereira G (2001). Medical surveillance from manufacturing site of diuron. Bayer AG, Agrochemical Centre Monheim, PF-E/REG, Gebaude 6100, D-51368 Leverkusen & Griffin (Europe) SA, Minervatstraat 8, B-1930 Zaventem.

Zapp Jr (1970). Skin primary irritation and sensitisation test in guinea pigs. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51443.

References Noted but not Reviewed

Andersen KJ, Leighty EG, Takahashi MT (1972). Evaluation of herbicides for possible mutagenic properties. J Agr Food Chem 20: 649-656.

Bentley KS (1995a). Mutagenicity testing of DPX-14740-194 (Karmex DF) in the Salmonella Typhimurium and Escherichia coli plate incorporation assay. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, 19714 USA. Report No: 51564.

Bentley KS (1995b). Mutagenicity testing of DPX-14740-200 (Karmex 500 SC) in the Salmonella Typhimurium and Escherichia coli plate incorporation assay. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, 19714 USA. Report No: 51565.

Beres E (1986). Acute inhalation toxicity test of Sadiuron 80 WP herbicide on Wistar rat. NEVIKI Research Institute for Heavy Chemical Industries. Veszprem.

Biegel LB (1995a). Mouse bone marrow micronucleus assay of DPX-14740-194 (Karmex DF). Lab: EI du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report No: 52016.

Biegel LB (1995b). Mouse bone marrow micronucleus assay of DPX-14740-200 (Karmex 200 SC). Lab: EI du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report No: 51566.

Bohme C & Ernst W (1965). Uber Den Stoffwechsel Von Harnstoff-Herbiciden Der Ratte. 2. Mitteilung. Diuron and Afalon. Fd Cosmet. Toxicol 3:797-802. Report No. Not stated.

Brown MA (1997). Hempel's Mille Dynamic 7170A: Magnusson-Kligman Maximisation test in guinea pigs for delayed skin sensitisation potential. Lab: Inveresk Research, Tranent, EH33 2NE, Scotland. Inveresk Report No: 15063.

Brown MA & Wilson JA (1997). Hempel's Seatech Antifouling 7820A: Primary skin irritation test in rabbits. Lab: Inveresk Research, Tranent, EH33 2NE, Scotland. Inveresk Report No: 14521.

Cox LR (1996). Mouse bone marrow micronucleus assay of DPX-M2574-43 (KROVAR IDF). Lab: EI du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19714. Report No: Not stated.

Dashiell (1982). Eye irritation test in rabbits. Report No: 51459.

Dupont (1955). Toxicity of 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Report No: 51575.

Du Pont (1980). Toxicological information. Report No: 51578.

Ernst W&Bohme C (1965). Uber Den Stoffwechsel Von Harnstoff-Herbiciden Der Ratte. 1. Mitteilung. Monuron und Aresin. Fd Cosmet. Toxicol 3:789-796. Report No. Not stated.

Evaluation of carcinogenic, teratogenic, and mutagenic activities of selected pesticides and industrical chemicals – Volume 1. Carcinogenic study. Report No: 51495.

Fahrig R (1974). Comparative mutagenicity studies with pesticides. Central Laboratory for Mutagenicity Testing of the German Research Association, Freiburg, FRG.

Filliben TA (1994a) Acute oral toxicity study with DPX-14740-194 (Karmex DF) in male and female rats. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No:51378.

Filliben TA (1994b). Acute oral toxicity study with DPX-14740-197 in male and female rats. Code: Karmex 90 DF. Lab: E I du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51380.

Filliben TA (1994c). Acute dermal toxicity study with DPX-14740-194 (Karmex DF) in rabbits. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51386.

Filliben TA (1994d). Acute dermal toxicity study with DPX-14740-197 in rabbits. Code: Karmex 90 DF. Lab: E I du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51387.

Filliben TA (1994e) Primary eye irritation study with DPX-14740-194 (Karmex DF) in rabbits. Report No: 51453.

Filliben TA (1994f). Primary dermal irritation study with DPX-14740-194 (Karmex DF) in rabbits. Report No: 51446.

Filliben (1994g) Primary eye irritation study with DPX-14740-197 in rabbits. Report No: 51452.

Filliben (1994h). Primary dermal irritation study with DPX-14740-197 in rabbits. Report No: 51445.

Finlay C (1993). Acute dermal toxicity study with DPX-14740-180 (Karmex DF) in rabbits. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51384.

Finlay C (1994). Acute oral toxicity study with DPX-14740-196 (Karmex 500 SC) in male and female rats. Lab: E I du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51379.

Finlay C (1994) Primary eye irritation study with DPX-14740-196 (Karmex 500 SC) in rabbits. Report No: 51454.

Finlay C (1994). Primary dermal irritation study with DPX-14740-196 (Karmex 500 SC) in rabbits. Report No: 51447.

Henry JE (1986). Eye irritation test in rabbits of Karmex DF for EPA pesticide registration. Report No: 51462.

Henry JE (1992). Eye irritation test in rabbits of Karmex DF for EPA pesticide registration (Revision 2). Report No: 51461.

Henry JE (1992). Eye irritation test in rabbits of Karmex DF for EPA pesticide registration (Supplement 1). Report No: 51460.

Henry JE (1986). Skin sensitisation test of Karmex DF in guinea pigs for EPA registration (Revision 1). Report No: 51451.

Innes JRM et al (1969). Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. J Nat Cancer Inst. 42: 1101-1114.

Jeang CL & Li GC (1980). Screening of pesticides for mutagenicity in the microbial system. II with mammalian microsomal activation. Natl Sci Counc Monthly ROC 8: 551-559.

Kalra SK & Chahal KS (1979). Degradation of diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea in cows. Ecotoxicology Environmental Safety 3: 362-368. Report No: 51568.

Kasa Z (1986a). Study on the acute toxicity of Sadiuron 80 WP herbicide Vol I. (Acute oral toxicity in rat, acute percutaneous toxicity in rat, acute eye irritant effect in rabbit, acute dermal irritant effect in rabbit). NEVIKI Research Institute for Heavy Chemical Industries. Institutional Report No 1940.

Kasa Z (1986). Study on the acute toxicity of Sadiuron 80 WP herbicide Vol II. (Acute skin irritation and sensitisation testing of diuron technical active ingredient on guinea pig). NEVIKI Research Institute for Heavy Chemical Industries. Veszprem.

Kasa Z (1986). Study on the acute toxicity of Sadiuron 80 WP herbicide. (Acute oral toxicity in mice). NEVIKI Research Institute for Heavy Chemical Industries. Veszprem.

Kehring B (2000). Arbeitsmedizinische erfahrungen mit diuron.

Kennedy GL (1980) Oral LD50 test in rats – EPA proposed guidelines. Code: IN-14,740-109. Lab/Sponsor: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51373.

Kennedy GL (1980) Acute skin absorption test on rabbits – LD50. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51383.

McRae LA (1995). Acute dermal toxicity to the rat of Hempel's Mille Dynamic 7170A. Report No: HMP 22f/940822/AC.

Moore (1994). Delayed contact hypersensitivity test (Buehler Method) with DPX-14740-196 (Karmex 500 SC) in guinea pigs. Report No: 51441.

Moore (1994). Delayed contact hypersensitivity test (Buehler Method) with DPX-14740-194 (Karmex DF) in guinea pigs (Revision 1). Report No: 51440.

Moore (1994). Delayed contact hypersensitivity test (Buehler Method) with DPX-14740-197 in guinea pigs. Report No: 51439.

Moriya M et al (1983). Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutation Res. 116, 185-216, 1983.

Oncogenicity of diuron (an explanation and summary of the following to publications. Report No: 51487.

Oncogenicity of diuron (1968). Report No: 51489.

O'Neill AJ (1993). Inhalation median lethal concentration (LC50) study with DPX-14740-181 (Karmex DF) in rats. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51466.

O'Neill AJ (1995a). Inhalation median lethal concentration (LC50) study with DPX-14740-198 in rats. Code: Karmex 90 DF. Lab: E I du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51464.

O'Neill AJ (1995b). Inhalation median lethal concentration (LC50) study with DPX-14740-200 (Karmex 500 SC) in rats. Lab: E I du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51463.

O'Neill AJ (1994). Inhalation median lethal concentration (LC50) study with DPX-14740-195 (Karmex DF) in rats. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51465.

Parcell BI (1997). Skin irritation to the rabbit of Hempel's Mille Dynamic 7170A. Lab: Huntingdon Life Sciences Ltd, PO Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Study Duration: September 1994. HRC Study No: HMP 23f/940806/SE. Guidelines: US EPA FIFRA 81-5. GLP/QA: Yes.

Romanelli (1993). Delayed contact hypersensitivity test (Buehler Method) with DPX-14740-180 (Karmex DF) in guinea pigs. Report No: 51442.

Sarver JW (1986) Median lethal dose (LD50) of Karmex DF in rats. Code: NB E-46497-1. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51370.

Sarver JW (1993) Acute oral toxicity study with DPX-14740-180 (Karmex DF) in male and female rats. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51377.

Sarver JW (1992) Primary eye irritation study with DPX-14740-174 (Karmex DF) in rabbits. Report No: 51457.

Sarver JW (1993) Primary eye irritation study with DPX-14740-174 (Karmex DF) in rabbits (Revision 1). Report No: 51456.

Sarver JW (1992) Primary eye irritation study with DPX-14740-175 (Karmex DF) in rabbits. Report No: 51455.

Sarver JW (1986). Skin irritation test in rabbits of KARMEX DF for EPA registration (Revision 1). Report No: 51450.

Sarver JW (1993). Primary dermal irritation study with DPX-14740-180 (Karmex DF) in rabbits. Report No: 51448.

Sarver JW (1986). Skin absorption LD50 or Karmex DF in rabbits for EPA pesticide registration. Code: NB E-46497-1. Lab: E I du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51382.

Sarver JW (1994). Acute oral toxicity study with DPX-14740-196 (Karmex 50 SC) in rabbits. Lab: E I du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, USA. Report No: 51385.

Stopps (1970). Primary irritation test on guinea pig and human skin. Report No: 51449.

Suzuki T&Casida JE (1981). Metabolites of diuron, linuron and methazole formed by liver microsomal enzymes and spinach plants. J Agric Fd Chem 29:1027-1033. Lab: Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of Califonia, Berkeley, California 94720. Report No: not stated.

Verheij ER, Greef van der J, Vos La GF, Pol van der W & Niessen WMA (1989). Identification of diuron and four of its metabolites in human postmortem plasma and urine by LC/MS with a moving-belt interface. J Anal Tox 13:8-12. Report no: not stated. GLP: no. Published: yes. [Bayer CropScience Pty Ltd; Sub: CR101-1, Vol 25 of 40]

Wilson JA & Brown MA (1997). Hempel's Seatech Antifouling 7820A: Acute oral toxicity (Limit) test in rats. Inveresk Report No: 14498.

Wnorowski G (1995a) Acute oral toxicity defined LD50. Lot #415135. Lab/Sponsor: Product Safety Labs, East Brunswick, NJ, USA / Griffin Corporation, Valdosta, GA, USA. Report No: 25558.

Wnorowski G (1995b) Acute dermal toxicity limit test. Lot #415135. Lab/Sponsor: Product Safety Labs, East Brunswick, NJ, USA / Griffin Corporation, Valdosta, GA, USA. Report No: 25559.

Wnorowski G (1995c) Acute inhalation toxicity limit test. Lot #415135. Lab/Sponsor: Product Safety Labs, East Brunswick, NJ, USA / Griffin Corporation, Valdosta, GA, USA. Report No: 25560.

Wnorowski G (1995) Direx 80 DF: Primary eye irritation. Report No: 25562.

Wnorowski G (1995) Direx 80 DF: Primary skin irritation. Report No: 25563.

Wnorowski G (1995). Direx 80 DF, Lot #415135: Dermal sensitisation test – Buehler Method. Report No: 25564.

Zapp, Jr. (1966) Eye irritation test. Report No: 51458.

Secondary/Other Citations*

Bucha HC & Todd CW (1951). 3-(p-Chlorophenyl)-1,1-dimethylurea; a new herbicide. Science 114: 493.

Butterworth BE, Conolly RB and Morgan KT. A strategy for establishing mode of action of chemical carcinogens as a guide for approaches to risk assessments. Cancer Letters 93: 129-146. 1995.

Cohen SM et al (1994). Mitogenic effects of propoxur on male rat bladder urothelium. Carcinogenesis 15:2593-2597.

Guilhermino L, Soares AM, Carvalho AP and Lopes MC (1998). Acute effects of 3,4-dichloroaniline on blood of male Wistar rats. Chemosphere 37: 619-32.

National Toxicology Program: NTP technical report on the toxicity studies of 3,3',4,4'-tetrachloroazobenzene (CAS No. 14047-09-7) administered by gavage to F344/N rats and B6C3F1 mice. Toxic Rep Ser 65:1-F6, 1998.

National Toxicology Program: NTP technical report on the toxicity studies of 3,3',4,4'-tetrachloroazoxybenzene (CAS No. 21232-47-3) administered by gavage to F344/N rats and B6C3F1 mice. Toxic Rep Ser 66:1-G4, 1998.

Schoket B & Vincze I. Dose-related induction of rat hepatic drug-metabolizing enzymes by diuron and chlorotoluron, two substituted phenylurea herbicides. Toxicology Letters, 50: 1-7, 1990.

Shaddock JG, Heflich RH, McMillan DC, Hinson JA and Casciano DA (1989). Pretreatment with mixed-function oxidase inducers increases the sensitivity of the hepatocyte/DNA repair assay. Environ Mol Mutagen 13: 281-288.

Stevens JT, Sumner DD. Herbicides. In: Hayes WJ Jr., Laws ER Jr. Handbook of pesticide toxicology. Volume 3: classes of pesticides. Toronto: Academic Press Inc., 1991:1317-408.

Valentovic MA, Yahia T, Ball JG, Hong SK, Brown PI and Rankin GO (1997). 3,4-dicholoroaniline acute toxicity in male Fischer 344 rats. Toxicology 124: 125-34.

Wang SW, Chu CY, Hsu JD & Wang CJ. Haemotoxic effect of phenylurea herbicides in rats: role of haemoglobin-adduct formation in splenic toxicity. Food and Chemical Toxicology, 31: 285-295, 1993.

*References cited within evaluated studies, correspondence or Committee records, or relevant literature, but not submitted to or assessed by the TGA.

ABBREVIATIONS AND ACRONYMS

Time	
d	Day
h	Hour
min	Minute
mo	Month
wk	Week
s	Second
yr	Year

Weight	
bw	Body weight
g	Gram
kg	Kilogram
µg	Microgram
mg	Milligram
ng	Nanogram
wt	Weight

Length	
cm	Centimetre
m	Metre
µm	Micrometre
mm	Millimetre
nm	Nanometre

Dosing	
id	Intradermal
im	Intramuscular
inh	Inhalation
ip	Intraperitoneal
iv	Intravenous
po	Oral
sc	Subcutaneous
mg/kg bw/d	mg/kg bodyweight/day

Volume	
L	Litre
mL	Millilitre
µL	Microlitre

Concentration	
M	Molar
ppb	Parts per billion
ppm	Parts per million

Clinical chemistry, haematology	
A/G	Albumin/globulin ratio
ALT	Alanine aminotransferase (SGPT)
AP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
BUN	Blood urea nitrogen
ChE	Cholinesterase
CPK	Creatine phosphatase (phosphokinase)
GGT	Gamma-glutamyl transferase
Hb	Haemoglobin
Hct	Haematocrit
LDH	Lactate dehydrogenase
LH	Luteinising hormone
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
NTE	Neurotoxic target esterase
PCV	Packed cell volume (Haematocrit)
PT	Prothrombin time
RBC	Red blood cell/erythrocyte
T_3	Triiodothyroxine
T_4	Thyroxine
TSH	Thyroid stimulating hormone (thyrotropin)
WBC	White blood cell/leucocyte
WBC-DC	White blood cells – differential count

Anatomy	
CNS	Central nervous system
GIT	Gastro-intestinal tract

Chemistry	
DMSO	Dimethyl sulfoxide
GC	Gas chromatography
GLC	Gas liquid chromatography

HPLC	High pressure liquid chromatography
MS	Mass spectrometry
RIA	Radioimmunoassay
TLC	Thin layer chromatography

Terminology

ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
GLP	Good Laboratory Practice
LOEL	Lowest Observed Effect Level
MRL	Maximum Residue Limit or Level
NOEL	No Observed Effect Level
NOAEL	No Observed Adverse Effect Level
OP	Organophosphorus pesticide

Organisations & publications

ACPH	Advisory Committee on Pesticides and Health
APVMA	Australian Pesticides and Veterinary Medicines Authority
CAC	Codex Alimentarius Commission
ECETOC	European Chemical Industry Ecology and Toxicology Centre
FAO	Food and Agriculture Organisation of the UN
FAISD	First Aid Instructions & Safety Directions
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
JECFA	FAO/WHO Joint Expert Committee on Food Additives
JMPR	Joint Meeting on Pesticide Residues
NCI	National Cancer Institute
NDPSC	National Drugs and Poisons Scheduling Committee
NHMRC	National Health and Medical Research Council
NOHSC	National Occupational Health & Safety Commission
NRA	National Registration Authority for Agricultural and Veterinary Chemicals
NTP	National Toxicology Program
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation