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

OFFICE OF PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

TXR#: 0050172

MEMORANDUM

DATE: March 19, 2002

SUBJECT: ***ENFROST* (Urea, End-Use Formulation):** Toxicology Chapter of the Tolerance Reassessment Eligibility Decision (TRED) for the Active Ingredient Pesticide, Urea.

EPA ID NO.: PC Code: 085702
DP Barcode: D274740
Submission Number: S596788
PRAT Case Number: 819300
Reregistration Case Number: 4096
CAS Registry Number: 57-13-6

FROM: Michelle M. Centra, Pharmacologist
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Michelle M. Centra

THRU: Catherine Eiden, Branch Senior Scientist
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for

TO: Joseph Nevola, Chemical Review Manager
Robert McNally, Branch Chief
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and

Rebecca Daiss, Risk Assessor
Reregistration Branch IV
Health Effects Division (7509C)

The Health Effects Division (HED) has conducted a reassessment of the available toxicity data for the frost protectant pesticide, urea. Since the Agency established a permanent exemption from the requirement of a tolerance for residues of urea in or on various agricultural commodities

(effective August 23, 1995) prior to passage of the Food Quality Protection Act (FQPA, 1996), a revised hazard characterization, including special sensitivity to infants and children is required.

This memorandum contains the toxicology chapter for urea tolerance reassessment eligibility decision (TRED) document. An electronic copy of this document is available and stored under the following Toxicology Record (TXR) Number: 0050172.

The following supporting documents used to generate the TRED toxicology chapter for urea are also included as attachments:

Health Effects Division Documents

1. Review of Six Acute Toxicity Studies and Two Literature Reviews on Urea. Review PP# 8F3662 (Memorandum: S. Stolzenberg, June 30, 1989).
2. Petition for use of Urea on crops as a frost protection agent. Request to waive certain toxicity data requirements. PP# 8F3662. (Memorandum: D. Ritter, February 24, 1989).
3. Urea (Enfrost); Reclassification of Six Acute Toxicity Studies from Supplementary to Guideline (Memorandum: R. Landolt, June 17, 1991).
4. Data waiver request for urea as an active ingredient for use as a frost protectant (Memorandum: J. Stewart, April 17, 2001).
5. *ENFROST* (Urea, End-Use Formulation): Re-evaluation of mammalian acute toxicity studies (OPPTS Test Guidelines 870.1100, 870.1200, 870.1300, 870.2400, 870.2500, and 870.2600) submitted by Unocal Corporation in Support of Registration. PC Code: 085702. DP Barcode: D277687. Submission Number: S596788. (Memorandum: M. Centra, March 10, 2002).

Additional Supporting Documents

6. Food and Drug Administration, Department of Health and Human Services, code of federal regulations (Parts 170 to 199, Revised as of April 1, 2001). 21 CFR Part 184.1923; Urea, page 549.
7. U.S. Environmental Protection Agency/Office of Pesticide Programs and Toxic Substances, Pesticide Fact Sheet (August 23, 1995).
8. Environmental Protection Agency, Federal Register Vol. 60, No. 163, Rules and Regulations. 40 CFR Part 180; Urea: Exemption From the Requirement of a Tolerance [PP-8F3662/R1176; FRL-4178-2] RIN 2070-AB78 (August 23, 1995).

cc (with attachments): M. Centra (HED/RRB III), C. Eiden (HED/RRB III), R. Daiss (HED/RRB IV), J. Nevola (SRRD/SRB), R. McNally (SRRD/SRB).

UREA

PC Code: 085702

Toxicology Disciplinary Chapter for the Tolerance Reassessment Document
TXR#: 0050172

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Prepared by: Michelle M. Centra, Pharmacologist
Date completed: October 2, 2001

INTRODUCTION

The active ingredient, urea, has GRAS (Generally Recognized as Safe) status as a direct food additive under Title 21 Code of Federal Regulations (CFR) 184.1923. The FDA affirmation of urea as safe was made by the Select Committee on GRAS Substances [a group of qualified scientists chosen by the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB)] in accordance with FDA guidelines. In the opinion of the members of this Committee " no evidence in the available information on urea demonstrates, or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future".

Urea is also exempt from the requirement of a tolerance under 40 CFR 180.1001(c) as an inert ingredient and sometimes as an active ingredient in formulations applied to growing crops or crops after harvest based upon the following considerations: (1) the amount of urea used as an active ingredient is similar to that permitted for the inert ingredient use (2) urea is a normal body constituent and is constantly being produced during amino acid and protein metabolism (3) urea is a naturally occurring crop/plant constituent that is found in commonly consumed foods (4) several grams of urea per kilogram body weight can be ingested by nonruminants, including man, without untoward effects (5) most of the nitrogen consumed in food is excreted in the form of urea. A 70 kg individual consuming a normal diet will excrete an average of 25 g urea daily (6) therapeutically, in humans, urea has been used as an osmotic diuretic to reduce blood pressure, intraoccular pressure and intracranial pressure, as a dermatologic agent to debride necrotic and infected tissues, as a topical anesthetic for the mouth and throat, in the treatment of sickle-cell anemia, and in neurosurgical procedures with few adverse effects and (7) urea has FDA-affirmed Generally Recognized as Safe (GRAS) status.

In 1995, the EPA granted a permanent exemption from the requirement of a tolerance for residues of the frost protectant urea in or on various raw agricultural commodities. Since this decision was made prior to the passage of the Food Quality Protection Act (FQPA, 1996), a revised hazard characterization that includes special sensitivity to infants and children is required for the urea Tolerance Reassessment Eligibility Decision (TRED) document.

HED's Toxicology Science Advisory Council (TOX SAC) met on March 22, 2001 to consider a request to reaffirm the toxicology data waivers granted in 1989 for the use of urea as a frost protectant on food crops (Memoranda: Ritter to Wilson, dated 2/23/89 and Stolzenberg to Rossi, dated 6/13/89). The TOX SAC examined the 1978 Monograph on urea by the FDA Select Committee on GRAS Substances of the Life Sciences Research Office (LSRO), Federation American Society of Experimental Biology (FASEB), as well as, the HED One Liners, and the 21 CFR Citation 184.1923, which affirms urea as GRAS as a direct human food ingredient. It was noted that the FDA GRAS affirmation was without limitations other than the current good manufacturing practice. There are no prior sanctions for this chemical. Based on the information

presented to the TOX SAC, the Council voted unanimously to reaffirm the toxicology data waivers, and to recommend that no further toxicity studies be required. The reaffirmed toxicology data waivers are listed in Table 1.

TABLE 1. HED REAFFIRMED TOXICOLOGY DATA WAIVERS	
Study Type	**Guideline Number**
90 Day Feeding Study in Rodents	870.3100
90 Day Feeding Study in Nonrodents	870.3150
21 Day Dermal Toxicity Study	870.3200
90 Day Dermal Toxicity Study	870.3250
90 Day Inhalation Toxicity Study	870.3465
Chronic Feeding Studies in Rodents and Nonrodents	870.4100
Carcinogenicity Studies in Two Mammalian Species	870.4200
Developmental Toxicity Studies in Rodents and Nonrodents	870.3700
Multigeneration Reproduction Study in Rodents	870.3800
Battery of Mutagenicity Studies	870.5100; 870.5300; 870.5385; 870.5375 and 870.5395
General Metabolism Study	870.7485

From the available animal studies and human exposure, the EPA has concluded that toxicity from exposure to urea is low. This conclusion agrees with that advanced in 1978 by the Select Committee on GRAS substances of the Life Sciences Research Office. The following hazard characterization includes an extensive review of literature toxicology data in numerous species, including man, to support the view that urea is safe in reasonably anticipated patterns of usage.

HAZARD CHARACTERIZATION

With the exception of six acute toxicity studies submitted by the Registrant for the Enfrost formulation, the urea toxicology data base is primarily comprised of the available literature data. These data are considered complete to assess the potential hazard to humans, including special sensitivity of infants and children.

Acute Toxicity

Ruminants are much more sensitive to urea than are nonruminants. The sudden ingestion of 116 g (about 230 mg per kg) by cattle or 10 g (about 160 mg per kg) by sheep, undiluted by feed, has resulted in labored breathing, tetanic spasms and prostration within 30 minutes.

Among nonruminants, the acute toxicity of urea appears to be relatively low. The lethal dose (LD_{50}) for an oral exposure in rats was 14,500 mg/kg which would be equivalent to a two pound ingestion of urea by an average size adult human. The acute toxicity of urea has also been evaluated in rabbits, cattle, sheep, dogs, guinea pigs, frogs, pigeons and ponies by oral, subcutaneous and intravenous exposures. Urea was slightly toxic in these mortality studies. Acute toxicity data are shown in Table 2.

TABLE 2. ACUTE TOXICITY OF UREA

Animal	Route of Administration	Results
Rabbit	Oral	LD_{50} = 5000 mg/kg
	Gavage	LD_{50} = 5000
	Subcutaneous	LD_{50} = 3000-9000
	Intravenous	LD_{50} = 7320
	Intravenous	LD_{50} = 6310
Cattle	Oral	MLD_{50} = 510
	Oral	MLD_{50} = 600-1080
Pony	Gavage	LD_{50} = 3310-3610
Sheep	Oral	MLD_{50} = 510
Dog	Subcutaneous	LD_{50} = 3000-9000
	Intravenous	LD_{50} = 3000
	Intravenous	MLD_{50} > 10,000
Guinea pig	Intravenous	LD_{50} = 4800
Frog	Subcutaneous	LD_{50} = 600-1000
Pigeon	Subcutaneous	LD_{50} = 16,000

LD_{50} = a statistically derived expression of a single dose of material that can be expected to kill 50 percent of the animals.

MLD_{50} = the median single dose of a material that can be expected to kill 50 percent of the animals.

No serious reactions have been recorded in humans with proper parenteral urea therapy. In man, intravenous administration of 1 g per kg body weight urea to reduce intracranial pressure may cause headaches (similar to those following lumbar puncture), nausea, vomiting, mental confusion, hyperthermia, nervousness, tachycardia and occasionally, fainting. However, these adverse effects can be minimized by slow infusion. Urea is also used orally as a diuretic in daily doses of 40 to 100 g (0.7 to 1.6 g/kg). Two to 3 g/kg body weight have been given orally to normal volunteers with no reported untoward effects, except significant diuresis.

The therapeutic effectiveness of urea in sickle-cell anemia is still controversial, but its use provides additional data on its possible acute toxicity. Massive doses of urea have been injected intravenously into patients during sickling crisis. The total dose of urea varied from 2.6 g/kg body weight injected over a 10-hour period to a maximal dose of 6.0 g per kg administered within 12 to 24 hours. The injection fluid was 10 or 15 percent urea dissolved in 10 percent invert sugar solution. Adverse effects were not serious, consisting mainly of diuresis, headache and vein irritation. However, the diuresis was considerable. In one instance, the urinary output over an 18-hour period was more than 28 liters.

In man, urea may cause redness and irritation to the skin. Urea is considered a mild skin irritant when applied to human skin intermittently (22 mg urea) over a 3 day period. With parenteral therapy, urea is more irritating to skin than mannitol, another popular osmotic agent. Although chemical phlebitis and thrombosis near the injection/infusion site are rare, urea may cause pain and some tissue injury if excessive bleeding occurs.

Many commercially available moisturizers contain urea (10-15 %) as an active ingredient and it is believed to be of importance in the prophylaxis and treatment of dry skin disorders. No systemic side-effects have been noted with use of skin moisturizers. All reported adverse effects were classified non-serious; some topical preparations caused disagreeable sensations such as smarting, stinging and itching immediately after application, however, these preparations did not cause skin irritation in the ordinary sense and usually did not cause damage to the skin barrier. The active ingredient, urea, has been shown to produce burning reactions on lesional forearm skin at concentrations similar to those present in creams.

Prolonged eye contact with high concentrations of urea may cause irritation (redness) and damage. Exposure of rabbit eyes to a saturated urea solution resulted in loss of epithelium from the cornea after five minutes contact and produced a grayness of the corneal stroma. One hour exposure to a 40% urea solution in rabbit eyes resulted in similar corneal effects with recovery in several weeks. Injection of 0.2 ml of a 10 M urea solution (0.12 grams) into the vitreous humor of rabbits caused inflammation, chorioretinitis and retinal degeneration.

Toxicity studies conducted with the Enfrost formulation (urea, 43.5% a.i.) submitted by the registrant show low toxicity following acute exposure (Table 3). Enfrost has a low order of acute toxicity via oral, dermal and inhalation routes (Toxicity Category III or IV) and produces slight irritation to the eyes and skin (Toxicity Category IV). Enfrost is not a dermal sensitizer.

TABLE 3. ACUTE TOXICTY PROFILE FOR ENFROST (Urea, 43.5% a.i.)				
Guideline	**Study Type (Date)**	**MRID**	**Results**	**Tox. Cat.**
870.1100 (§81-1)	Acute Oral-Rat (5/11/88)	40733304	LD_{50} > 5000 mg/kg	IV
870.1200 (§81-2)	Acute Dermal-Rabbit (5/11/88)	40733305	LD_{50} > 2000 mg/kg	III
870.1300 (§81-3)	Acute Inhalation-Rat (5/11/88)	40733301	LC_{50} > 4.8 mg/L	III
870.2400 (§81-4)	Primary Eye Irritation-Rabbit (5/11/88)	40733302	Slight eye irritant	IV
870.2500 (§81-5)	Primary Dermal irritation-Rabbit (5/11/88)	40733306	Slight dermal irritant	IV
870.2600 (§81-6)	Dermal Sensitization-Guinea pig (5/11/88)	40733303	Non sensitizer	N/A

Subchronic Toxicity

Toxicity to urea is dependent on species, body size, nutritional status, rate of feeding and nature of the diet. Most of these studies have been conducted with ruminants. The American Feed Control Officials recommend that the amount of urea fed to cows not exceed 3 percent of the total grain ration, which represents about 0.45 g/kg/day. Various reports indicate that sheep can ingest 50 to 100 g (about 0.8 to 1.6 g/kg) urea daily with no harmful effects when properly mixed with feed.

In a subchronic toxicity study, urea produced no severe toxicity in dogs injected subcutaneously with 30-40 ml/kg/day of 10% urea solution for 45 days. With plasma levels ranging from 200-700 mg/100 ml (10-30 fold above normal), the only clinical symptoms observed were drowsiness and diuresis. Necropsy indicated no adverse organ pathology. Therefore, urea was not considered an important uremic toxin in dogs with normal renal function. However, studies of nephrectomized dogs in which urea levels of 540-1690 mg/100 ml of extracellular fluid were maintained for 10 days by means of intermittent peritoneal lavage, severe uremic signs, such as weakness and anorexia followed by retching, vomiting, diarrhea, reduced body temperature and culminating in deep torpor or coma were evident.

Rats fed rations containing 2 to 25 percent urea (2- 25 g/kg body weight daily) for periods up to 190 days showed systemic toxicities. Even at the lower levels of urea ingestion, weight loss and suppression of sexual function resulted. Rats receiving14 percent urea in their diet and deprived of water died within a few days. If water were allowed, animals survived for 20 to 76 days when fed the 20 percent urea supplement and 12 days when fed the 25 percent urea supplement.

Anemia and renal hypertrophy were also observed in some these animals. However, it is difficult to interpret these findings because of the number of rats tested per treatment group was small (often 1 to 3) and no data were given on the actual food intake. The extreme weight loss observed in rats suggests that starvation was most likely the result of decreased palatability of the animal feed containing urea.

In contrast, severe forms of uremia are not manifested in dialysis patients with blood urea concentrations above 300 mg/100 ml. High blood concentrations of 181 to 600 mg urea/100 ml were maintained by intermittent dialysis in three patients suffering from advanced renal failure for periods of 7 to 90 days. When the urea concentration was kept below 300 mg per 100 ml, no untoward effects were noted although this level is about 10 times greater than normal. Concentrations above 300 mg per 100 ml were associated with malaise, vomiting, bleeding tendency and headache. However, the more severe gastrointestinal, cardiovascular, mental and neurologic changes of uremia were not observed.

In eight patients with sickle cell disease, 40 to120 g (0.6 to 2.0 g/kg) urea was administered orally in divided doses each day for periods of 3 weeks to 9 months. The blood urea concentrations of the patients approximately doubled during the test periods. While the patients were ingesting urea, there was a slight decrease in blood volume, probably resulting from the chronic osmotic diuresis induced by the urea. The most obvious effects of the urea intake were thirst and diuresis and two patients were unable to complete the study because of nausea and vomiting.

Chronic Toxicity and Carcinogenicity

No toxicities from urea have been reported in humans after chronic exposures. One year feeding studies in male and female C57B1/6 mice and Fisher 344 rats reported no evidence of treatment-related cancer at doses up to 4.5% of the diet. Slight increases in the incidence of lymphomas occurring in mid-dose female mice as well as interstitial cell adenomas of the testes occurring in high-dose male rats, were not considered biologically significant in this study.

Earlier studies also indicated no evidence of urea tumorigenicity. Doses of 10 to 50 mg urea (0.5 - 2.5 g/kg were injected subcutaneously in mice (Strain A) over a period of 11 months. No tumors were evident after 15 months. Weekly intraperitoneal injections of 400 mg/kg urea administered over a 13 week interval produced no lung adenomas in the sensitive mouse strain (Strain A).

Developmental and Reproductive Toxicity

In a developmental toxicity study, pregnant Wistar rats receiving a twice-daily dose of 25 g/kg urea by gastric intubation for 14 days produced healthy offspring with no reported evidence of teratogenic effects. Additionally, pregnant cows, which recovered from urea toxicity, exhibited no effects on reproductive performance nor were the calves affected. No effect on the number of

calves born, birth weight, weaning weight of calves or rebreeding performance was observed in these animals when treated acutely with urea (0.44 g/kg) and kept under regular management for 12 months. However, immersion of frog eggs in 1.25% urea and injection of chick embryos with 50-900 mg urea have produced some evidence of neural, vascular or cardiac abnormalities.

Urea has been evaluated in monkeys and humans for its ability to induce abortion. In humans, intra-amniotic injection of 80 grams "Ureaphil"/210 ml in 5% dextrose was effective in inducing abortion at 14 weeks without adverse effects to the mother. The mode of action is similar to the hyperosmolar effect of large doses of hypertonic saline and dextrose where a highly localized hyperosmolar solute passes from the amniotic fluid into the fetus causing death. Such high intrauterine exposures would not occur from environmental exposure to urea. Urea is currently classified by FDA in category C for therapeutic use ("Safety for use during pregnancy has not been established").

Mutagenicity

Several *in vitro* studies have reported that urea is associated with chromosomal aberrations in human leukocytes, hamster fibroblasts and lung cells. All of these studies were conducted with urea concentrations ranging from 50 mM to 8 M. At physiological levels (1mM), urea causes no chromosome effects. However, at concentrations of urea greater than or equal to 50mM, the production of chromosome fragmentation is probably due to a non-specific, hyperosmolarity effect on cell division and not a direct effect of the urea molecule. Sodium phosphate, another normal body fluid constituent also produces chromosomal damage at 50 mM concentrations.

Absorption, Metabolism, and Excretion

Urea is extremely soluble in water and oral doses are rapidly absorbed and distributed through the most body tissues and fluids, in proportion to their water content. However, the penetration of urea into fatty tissue such as the brain is lower than for most other tissues. The colon has been reported to be relatively impermeable to urea. When urea solutions were introduced into the colon in men, urea concentration in the blood remained unchanged.

In pregnant rats injected subcutaneously with urea, it was found that not only had urea penetrated rapidly into maternal tissues and organs but that it also readily passed through the placenta. Within 30 minutes after injection, the urea content of maternal muscle and liver had increased approximately threefold over the control value, and the fetal concentration had doubled. Two hours after injection, the fetus and the maternal liver and muscle contained equal concentrations of urea. When sheep were fed 40 g of urea with 40 g of glucose, the urea content of portal blood doubled within 15 minutes.

In man, too, the absorption of urea is very rapid. Blood urea concentration was found to reach a peak, generally within 30 minutes after oral administration. Similar results were reported in human volunteers; serum urea levels doubled within 20 minutes after receiving 30 g of urea by

mouth (about 0.5 g/kg). A maximum level of 94.6 mg/100 ml was reached within 40 minutes in treated volunteers compared to 36.4 mg/100 ml in untreated volunteers. The normal concentration of urea in the blood plasma of man is approximately 0.26 mg/ml (range: 20 to 30 mg/100 ml).

Urea is formed metabolically through a cyclic mechanism. Free ammonia arising from the oxidative deamination of glutamate in liver mitochondria combines with carbon dioxide to form carbamoyl phosphate. The carbamoyl group is transferred to ornithine to form citrilluline, which in turn reacts with aspartate to produce arginosuccinate. This is hydrolyzed enzymatically to liberate free arginine and femorata. The femorata returns to the pool of tricarboxylic acid cycle intermediates, while the arginine is cleaved by anginose to produce urea and ornithine.

The so-called aerolytic animals excrete urea as the major end-product of amino acid metabolism. Included in this group are mammals, elasmobranch, amphibia and Chelonia. Genetic deficiency of any of the enzymes required in the urea cycle produces protein intolerance, elevated amounts of blood ammonia, metabolic disturbances, neurological symptoms and brain damage. The development of the urea cycle enzymes in the fetus varies with the species. The pig fetus is able to synthesize urea at a very early stage, but the rat fetus acquires this ability only at a later period.

Urea is an end product of protein and ammonia metabolism in humans and a 70 kg adult excretes urea in the amount of 25-30 g/day (350-420 mg/kg/day). An individual consuming a high protein diet will excrete about 90 percent of the dietary nitrogen as urea whereas the percentage excreted as urea is less with a highly restricted nitrogen intake. The ability of the kidney to remove urea from the blood provides one method of assessing renal function, or more specifically, glomerular filtration capacity. However, the measurement of blood urea nitrogen (BUN) may be affected by poor nutrition and hepatotoxicity, which are common effects of many toxicants. Glomerular filtration rate can be also be determined by the renal clearance of creatinine, inulin, p-aminohippuric acid (PAH) and phenolsulfophthalein.

Urea had long been used as a dietary supplement for ruminants and in 1949, it was demonstrated that urea could serve as a nitrogen source in weanling rats as well. Similar utilization of urea has now been shown in rabbit, chick, pig, horse, and man. Bacterial action in the gastrointestinal tract, particularly in the colon, produces ammonia which is absorbed and mixed with the metabolic pool of nitrogen, where some may be utilized for protein synthesis. Urea nitrogen can contribute part of the amino acid requirements in man when the diet provides sufficient glucose for nonessential amino acid synthesis and utilization of urea nitrogen has been demonstrated both in malnourished children and adults. It has been estimated that the potential contribution of urea or ammonium salts to protein synthesis in man is less than 10 percent.

Therapeutic Uses

Urea is approved for several therapeutic uses in humans with relatively few toxicities. Urea is used primarily as an osmotic agent for inducing diuresis and reducing intraoccular and intracranial pressure (Ureaphil, 30% urea solution). Intravenous doses of 1-1.5 g/kg urea (30% urea solution) are considered optimal for neurosurgical procedures with no adverse effects. In addition, urea is approved by the FDA for topical use as (i) an anesthetic for the treatment of mouth and throat inflammation (10-15% urea gel, liquid or solution), (ii) a topical agent to debride necrotic and infected tissues, i.e. fingernails and toenails (2-40% formulations), and (iii) an active ingredient formulated within moisturizers (10-15% urea) for use in the prophylaxis and treatment of dry skin disorders. It is also used in the treatment of sickle-cell anemia and to ammoniate dentrifices as well as a basic ingredient in the synthesis of medically important compounds such as barbiturates and urethanes.

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ATTACHMENT 1

Review of Six Acute Toxicity Studies and Two Literature Reviews on Urea.
Review PP# 8F3662 (Memorandum: S. Stolzenberg, June 30, 1989).

An electronic version of this document is not available. See the hard copy file.

CASWELL FILE

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

JUN 30 1989

007297

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of Six Acute Toxicity Studies and Two Literature Reviews on Urea. Review PP #8F3662.

Caswell No. 902 HED TOX Project No. 8-1038
Record Nos. 225,957 I.D. No. 612 A
229,100
CAS #57-13-6

FROM: Sidney Stolzenberg, Ph.D. SJStolzenberg 6/13/89
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and

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Registration Division (H7509C)

THRU: Michael Ioannou, Ph.D. M.F. Ioannou 6-12-89
Section Head, Review Section I
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Health Effects Division (H7509C)

and

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Acting Branch Chief, Tox. Branch II (HFAS)
Health Effects Division (H7509C)

Applicant: Unocal Corporation
Los Angeles, CA 90051

ACTION REQUESTED:

1. Screen data of 6 acute toxicity studies for being acceptable and plan a meeting with the applicant regarding the results of the screening.

2. Examine a literature review on urea of 1978 by the FDA Select Committee on GRAS Substances of LSRO and a literature review on the evaluation of the health aspects of urea as a food ingredient, performed by the applicant.

17

3. Review 8F3662, which is a Petition for an Exemption from Requirement of a Tolerance.

4. This submission contains a request for a waiver of all additional toxicology studies required in 40 CFR 158, which includes the following:

90-Day Feeding in rodent and non-rodent (82-1).
21-Day Dermal (82-2).
90-Day Dermal (82-3).
Cronic Feeding in rodent and non-redent (83-1).
Oncogenicity Study in rat and mouse (83-2).
Teratogenicity in 2 species (83-3).
Reproduction, 2-generation (83-4).
Mutagenicity Battery (84-2 thru 84-4).
General Metabolism (85-1).

The bases for the waiver request, as cited by the applicant, are as follows:

a. Urea has a GRAS status with FDA as a direct food additive under 21 CFR 184.1923.

b. Urea is exempt from the requirement of a tolerance as an inert or sometimes active ingredient in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest, under 40 CFR 180.1001 (c).

c. The amount of urea proposed for application to individual crops is equivalent to the level permitted as inert ingredient in pesticide formulations and the level commonly used in fertilizer applications.

d. Urea is a constituent of animal tissue and body fluids and is the excretory end product of protein metabolism; about 25 grams/day is the average excretion rate in urine by a 70 kg person.

e. Urea also occurs naturally in plants; e.g. up to 4.5% of the nitrogen in oats. Up to 15% of total nitrogen in young plants and 5% in mature plants is non-protein nitrogen, much of which is in the form of urea.

f. A copy of the 1978 monograph by the FDA Select Committee on GRAS Substances for urea is contained in this submission by the applicant. The Committee concluded that there is no evidence that demonstrates or suggests reasonable grounds to suspect a hazard to the public when urea is used at levels that are presently now current or that might be reasonably expected in the future.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Composition Listed for Unocal Plus (Confidential):

	Percent
Urea (active ingredient)	43.7
[redacted]	

Amount of Active Ingredient Listed for Enfrost: 42.9%
Amount of Inert Ingredients Listed for Enfrost: 57.1%

The product was previously known as **Unocal Plus** but is presently being renamed **Enfrost.** The composition of active ingredient (urea) and inert materials appear to be very similar or identical. The urea content listed for Unocal Plus is 43.7% whereas the urea content listed for Enfrost is 42.9%, a difference of only 0.8% if this is actually true.

The following is a list of proposed usages of Enfrost for the purpose of reducing frost damage to crops.

Crop	Gal./Acre
Deciduous Tree Crops (including almond, apple, apricot, cherries, fig, nectarines, peach, pistachio, prune)	4
Deciduous Tree Crops (walnut)	3
Deciduous Tree Crop (plum)	3
Nondeciduous Tree Crop (avocado, grapefruit, lemon, lime, olive, orange, tangerine)	15
Specialty Crops (Artichokes, strawberries)	5
Vegetable Crops (asparagus, brussel sprout, carrot, celery, cucumber, onion, squash)	5
Cole Crops (Broccoli, cauliflower)	10-20
Leafy Vegetables (bok choy, lettuce, napa cabbage)	5
Peppers	5
Field Crops (alfalfa, beans, corn, safflower, sorghum)	5-10
Vine Crops (boysenberry, grape, kiwifruit, rasberry)	5
Melons (cantelope, casaba, crenshaw, Persian)	5
Nursery Crops	5
Seed Crops	5

The above applications may be repeated if necessary after a period of 4 to 7 days if cold weather persists.

RECOMMENDATIONS

1. Five of the 6 acute toxicity studies submitted in support of Enfrost as a frost protection agent on foods were classified as Core Sepplementary. In each of these studies, the registrant failed to specify the purity of the the test substance. The following is a list of the studies and classification of each one.

Study, Route, (Guideline)	Core Classif.	MRID No.
Acute, oral, (81-1)	Supplementary	407333-04
Acute, dermal toxicity (81-2)	Supplementary	407333-05
Acute, inhalation (81-3)	Minimum	407333-01
Primary eye irritation (81-4)	Supplementary	407333-02
Primary dermal irritation (81-5)	Supplementary	407333-06
Dermal sensitization (81-6)	Supplementary	407333-03

The registrant should also specify if the urea composition in the new product is 43.7 % as listed for Unocal Plus, or 42.9% as listed for Enfrost.

All of the above studies are upgradeable when the information required is supplied.

The following is a summary of the Toxicology Data and Toxicity Categories for the six acute studies which is applicable to the acute inhalation study and will be applicable to the 5 studies classified as Supplementary when upgraded.

	Results	Tox. Cat.
1). Acute oral LD_{50}, rat.	>5 g/kg	IV
2). Acute inhalation, LC_{50}, rat	>4.8 mg/L/4 hr	III
3). Primary Eye Irritation, rabbit		IV
P.I.S. at 1 hour in unwashed eye:	3.2	
at 48 hour in unwashed eye:	0	
4). Acute dermal toxicity, rat	>2 g/kg	III
5). Primary dermal irritation, rabb.		IV
P.I.S. at 30-60 minutes:	0.2	
at 72 hours:	0	
6). Dermal sensitization, guinea pig. Not a dermal sensitizer.		

2. The two literature reviews cited above, presented by the applicant with this submission, supports the view that urea is safe when used in food commodities at reasonably anticipated patterns of its usage.

3. Urea was considered as GRAS, according to FDA guidelines and is therefore exempt from premarketing clearance required by FDA for food additives under 21 CFR 184.1923. Urea is exempt from the requirement of a tolerance as an inert and sometimes active ingredient in pesticide formulations when applied to crops or to

raw agricultural commodities after harvest under 40 CFR 180.1001(c).

4. PP #8F3662, the petition for use of urea on crops as a frost protection agent and the request for a waiver on the toxicity studies listed above, have already been approved (See memo of D. Ritter to E. Wilson, dated Feb. 24, 1989).

5. [redacted] listed as an inert ingredient in the amount of [redacted] of the total product, is not listed in 40 CFR 180.1001, exemptions from tolerance on foods. A request for exemption should be submitted by the applicant.

007297

Reviewed by: Sidney Stolzenberg, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C) Sidney Stolzenberg 6/12/89
Secondary Reviewer: Michael Ioannou, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C) J.M.I. 6-13-89

DATA EVALUATION REPORT

STUDY TYPE: Literature Review

Caswell No. 902

TEST MATERIAL: 81-1

HED Project No. 8-1038

TEST MATERIAL: Urea

MRID No. 407333-07

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TITLE OF REPORT: Evaluation of the Health Aspects of Urea as a Food Ingredient

AUTHOR(S): Life Science Research Office (Literature Search performed for FDA under Contract No. FDA 223-75-2004).

REPORT ISSUED: 1978

CONCLUSIONS AND SUMMARY

Urea is considered GRAS by FDA (21 USC 321 (s)). A huge body of world literature pertaining to the health and safety aspects of using urea in food, supports the conclusion that urea is safe in common food use at reasonably anticipated patterns of consumption. The conclusion for recognition of urea as safe was made by the Select Committee on GRAS Substances of Life Science Research Office (LSRO) in accordance with FDA guidelines.

A brief review on background information which includes medical usage, manufacturing usage and consumer exposure data, are given. This report also includes an extensive review of toxicology data in numerous species, including man, to support the view that urea is safe in reasonably anticipated patterns of its usage.

U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-288 673

Evaluation of the Health Aspects of Urea as a Food Ingredient

Federation of American Societies for Experimental Biology, Bethesda, MD

Prepared for

Food and Drug Administration, Washington, DC Bureau of Foods

1978

SCOGS-103

EVALUATION OF THE HEALTH ASPECTS OF UREA AS A FOOD INGREDIENT

1978

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 223-75-2004

LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
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9650 Rockville Pike
Bethesda, Maryland 20014

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15. Supplementary Notes

16. Abstract (Limit: 200 words)

This report, by a group of qualified scientists designated the Select Committee on GRAS Substances (SCOGS), provides an independent evaluation of the safety of urea when used in foods at present or projected future levels.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

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c. COSATI Field/Group

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Bethesda, Maryland 20014

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NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

Kenneth D. Fisher

Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office
FASEB

CONTENTS

I. INTRODUCTION

This report concerns the health aspects of using urea as a food ingredient. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1973.* To assure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, an announcement was made in the Federal Register of June 13, 1978 (43 FR 25487-25489) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the health aspects of using urea as a food ingredient. The Select Committee received no requests for such a hearing on urea.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321 (s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (2) [21 CFR 170.3 and 170.30] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. These sections of the Code also indicate that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA (2) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance with

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*The document (PB-241 971/1) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.

FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. While the Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Committee, there are insufficient data upon which to base a conclusion. The Committee is aware that its conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on urea and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act.

II. BACKGROUND INFORMATION

Urea, $CO(NH_2)_2$ is the diamide of carbonic acid. It is a white, odorless, somewhat hygroscopic, crystalline solid. On standing, it may gradually develop a slight ammoniacal odor. It is highly soluble in water, glycerol and hot alcohol, but almost insoluble in chloroform and ether (3). Food grade urea is not listed in the Food Chemicals Codex (4). The U.S. Pharmacopeia specifies a purity of at least 99 percent with not more than 20 ppm of heavy metals, 100 ppm of sulfate, 70 ppm of chloride and 400 ppm of alcohol insoluble matter (5).

Several million tons of urea are produced annually in the United States, the bulk of which is used in agriculture as a slow release fertilizer and as a feed supplement. Its major medical role is to reduce intraocular and intracranial pressure. It has also been used in the treatment of sickle cell anemia, as a diuretic, as a topical antiseptic, and to ammoniate dentifrices (3, 6). It is a basic ingredient in the synthesis of medically important compounds such as barbiturates and urethanes (3).

Urea is used in the manufacture of dyes, fire retardant paints, plasticizers, and stabilizers for explosives. Upon reaction with formaldehyde, it forms resins which have broad applications as plastics and adhesives. These urea-formaldehyde resins are employed as bonding and adhesive agents for plywood, as laminating and protective coatings, and as paper and fabric modifiers. Such resins were the first commercially important products to achieve crease resistance and other desirable properties in cellulosic fabrics. They are used extensively in treating and coating paper to increase the wet strength and the general utility of paper products (7).

Urea appears among substances that are generally recognized as safe in the Code of Federal Regulations (2) for use in cotton and cotton fabrics in dry food packaging (21 CFR 182.70) and in paper and paperboard products (21 CFR 182.90). Unpublished GRAS authorizations include its use in foods, syrups for flavoring milk, chewing gum, vitamin and mineral preparations, as a marker in whiskey, and as a solubilizing agent for riboflavin (8). It is deemed to be generally recognized as safe by the Internal Revenue Service as food for yeast in wine production, with the amount used not to exceed 2 pounds per 1000 gallons (9) [27 CFR 240.1051]. Urea is a regulated food additive for use as a component in cellophane for packaging food (21 CFR 177.1200), in side seam cements for food containers (21 CFR 175.300) and as a plasticizer (in the form of the sodium nitrate-urea complex) in glassine and greaseproof paper for packaging dry foods (21 CFR 176.320).

III. CONSUMER EXPOSURE DATA

Urea is a natural constituent of many common foodstuffs. Oats may contain 4.5 percent of their total nitrogen content as urea and oil seed meals about 0.25 percent. Up to 15 percent of the total nitrogen of young plants and about 5 percent of the mature plants is nonproteinaceous and much is in the form of urea (10). Urea is a normal constituent of animal tissues and fluids and is ingested in small amounts when meat is consumed.

No data are available on the intake of urea resulting from its addition to food. A National Research Council subcommittee investigating the extent to which GRAS substances are added to food did not include urea in its survey of the food industry (11). No listing is shown for urea in the Handbook of Food Additives (12) which gives the usual levels of addition of many GRAS substances to food.

Approximately 4.2 million tons of urea were produced (13) and imported (14) in the United States in 1973, the latest date for which complete data are available. The Select Committee estimates that approximately 90 percent was used as feed supplements or fertilizers, leaving about 400,000 tons (360 million kg) for all other purposes. If all the urea not utilized for animal feed or fertilizer were added to food for human consumption, the per capita addition would be no more than 5 g daily. However, most of the urea not utilized for fertilizer or feed is used for the purposes listed in the previous section, especially for the production of urea-formaldehyde resins. Although no data are available on this point, it is believed that only a very small amount is used as a direct or indirect food ingredient.

IV. BIOLOGICAL STUDIES

Absorption, metabolism and excretion

Urea is extremely soluble in water and oral doses are rapidly absorbed and distributed through the body's tissues and fluids, in proportion to their water content (15, 16). The penetration of urea into fatty tissue such as the brain is lower than for most other tissues (17).

When sheep were fed 40 g of urea with 40 g of glucose, the urea content of portal blood doubled within 15 minutes (18). In man, too, the absorption of urea is very rapid. Archer and Robb (19) found the blood urea concentration to reach a peak, generally within 30 minutes after oral administration. Similar results were obtained by Shannon et al. (20) who reported that the serum urea levels of human volunteers doubled within 20 minutes after receiving 30 g of urea by mouth (about 0.5 g per kg). A maximum level of 94.6 mg per 100 ml (control 36.4 mg per 100 ml) was reached within 40 minutes. Luck and Engle (21) injected pregnant rats subcutaneously with urea and found that it not only penetrated rapidly into maternal tissues and organs but that it also readily passed through the placenta. Within 30 minutes after injection, the urea content of the maternal muscle and liver had increased approximately threefold over the control value and the fetal concentration had doubled. Two hours after injection, the fetus and the maternal liver and muscle contained equal concentrations of urea.

The colon has been reported to be relatively impermeable to urea (22). When urea solutions were introduced into the colon in men, urea concentrations in the blood remained unchanged.

Urea is formed metabolically through a cyclic mechanism first postulated in 1932 by Krebs and Henseleit (23). Free ammonia arising from the oxidative deamination of glutamate in liver mitochondria combines with carbon dioxide to form carbamoyl phosphate. The carbamoyl group is transferred to ornithine to form citrulline, which in turn reacts with aspartate to produce arginosuccinate. This is hydrolysed enzymatically to liberate free arginine and fumarate. The fumarate returns to the pool of tricarboxylic acid cycle intermediates, while the arginine is cleaved by arginase to produce urea and ornithine.

The so-called urotelic animals excrete urea as the major end-product of amino acid metabolism. Included in this group are mammals, elasmobranchs, amphibia, and chelonia (24). Genetic deficiency of any of the enzymes required in the urea cycle produces protein intolerance, elevated amounts of blood ammonia, metabolic disturbances, neurological symptoms and brain damage (25). The development of the urea cycle enzymes in the fetus varies with the species. The pig fetus is able to synthesize urea

at a very early stage, but the rat fetus acquires this ability only at a later period (26).

The normal range of urea in the blood plasma of man is 20 to 30 mg per 100 ml and a 70 kg adult excretes about 30 g daily. An individual consuming a high protein diet will excrete about 90 percent of the dietary nitrogen as urea. The percentage excreted as urea is less with a highly restricted nitrogen intake. The ability of the kidney to remove urea from the blood provides a measure of kidney function, or more specifically, of glomerular filtration capacity (27).

Urea has long been used as a dietary supplement for ruminants (28, 29) and in 1949, Rose et al. (30) demonstrated that it could serve as a nitrogen source in weanling rats as well. Similar utilization of urea has now been shown in the rabbit (31), chick (32), pig (33), horse (34), and man (35-37). Bacterial action in the gastrointestinal tract, particularly in the colon, produces ammonia which is absorbed and mixed with the metabolic pool of nitrogen, where some may be utilized for protein synthesis. Utilization of urea nitrogen has been demonstrated both in malnourished children (36) and adults (37). Gallina and Dominguez (38) report that urea nitrogen can contribute part of the amino acid requirements in man when the diet provides sufficient glucose for nonessential amino acid synthesis. Picou and Phillips (36) estimate that in man the potential contribution of urea or ammonium salts to protein synthesis is less than 10 percent.

Acute toxicity

Ruminants are much more sensitive to urea than are nonruminants. The sudden ingestion of 116 g (about 230 mg per kg) by cattle or 10 g (about 160 mg per kg) by sheep, undiluted by feed, has resulted in labored breathing, tetanic spasms and prostration within 30 minutes (28).

Among nonruminants, the acute toxicity of urea appears to be relatively low. Unfortunately, much of the available data is old and the experimental conditions vague. Acute toxicity data are shown in Table 1.

In man, the recommended dose to reduce intraocular and intracranial pressures is 1 g per kg body weight administered intravenously. Nausea, vomiting, mental confusion, hyperthermia, nervousness, and tachycardia may result but can be minimized by slow infusion. Urea may also be used orally as a diuretic in daily doses of 40 to 100 g (0.7 to 1.6 g per kg) (49). Two to 3 g per kg body weight have been given orally to normal volunteers with no reported untoward effects (50).

TABLE I

Acute Toxicity of Urea

Animal	Route	Index	Dose	Reference
			mg/kg	
Rabbit	oral (?)	LD_{50}	5000	40
	gavage	LD	5000	41
	S.C.	LD	3000-9000	42
	I.V.	LD	7320	43
	I.V.	LD	6310	44
Cattle	oral	MLD	510	18
	oral	MLD	600-1080	47
Pony	gavage	LD	3310-3610	45
Sheep	oral	MLD	510	48
Dog	S.C.	LD	3000-9000	42
	I.V.	LD	3000	42
	I.V.	MLD	>10,000	39
Guinea pig	I.V.	LD	4800	46
Frog	S.C.	LD	600-1000	42
Pigeon	S.C.	LD	16,000	42

The therapeutic effectiveness of urea in sickle cell anemia is still controversial, but its use provides additional data on its possible acute toxicity. Massive doses of urea have been injected intravenously into patients during sickling crises. The total dose of urea varied from 2.6 g per kg body weight injected over a 10-hour period (51) to a maximal dose of 6.0 g per kg administered within 12 to 24 hours (52). The injection fluid was 10 or 15 percent urea dissolved in 10 percent invert sugar solution. The investigators concluded that the rapid infusion of large amounts of urea was not superior to invert sugar alone in shortening the crisis episodes. The side effects were not serious, consisting mainly of diuresis, headache, and vein irritation. The diuresis was considerable. In one instance, the urinary output over an 18-hour period was more than 28 liters.

Short-term studies

Chronic toxicity to urea is dependent on species, body size, nutritional status, rate of feeding and nature of the diet. Most of these studies have been conducted with ruminants. The American Feed Control Officials (53) recommend that the amount of urea fed to cows not exceed 3 percent of the total grain ration, which represents about 0.45 g per kg per day. Various reports indicate that sheep can ingest 50 to 100 g (about 0.8 to 1.6 g per kg) urea daily with no harmful effects when properly mixed with feed (54).

Richet and Maret (55) fed rats for periods up to 190 days with rations containing from 2 to 25 percent urea (about 2 to 25 g per kg body weight daily). Even at the lower levels of urea ingestion, weight loss and suppression of sexual function resulted. Rats receiving 14 percent urea in their diet and deprived of water died within a few days. If water were allowed, they survived for 20 to 76 days at the 20 percent level and 12 days with the 25 percent supplement. Anemia and renal hypertrophy were also observed in some animals. It is difficult to evaluate these findings for the number of animals in each series was small (often 1 to 3) and no data are given on the actual food intake. The extreme weight loss of the rats suggests that inanition was likely.

Balestri et al. (56) injected subcutaneously 3000 to 4000 mg urea per kg into 12 unilaterally nephrectomized dogs every 8 hours for a period of 45 days. Plasma urea levels were maintained between 200 and 700 mg per 100 ml. Hematocrit and platelet counts were made in some animals, electroencephalographic recordings in some and measurements of spontaneous movement in others. Except for a mild drowsiness and increased diuresis, the results of all measurements were essentially normal.

Grollman and Grollman (57), however, claimed that many of the signs encountered in uremia were due to the effect of accumulated urea. They believed the presence of high urea levels induced changes in tissue electrolytes which were at least partly responsible for the observed toxic

effects. The investigators maintained concentrations of 540 to 1690 mg urea per 100 ml extracellular fluid in nephrectomized dogs by means of intermittent peritoneal lavage. The first signs of toxicity were weakness and anorexia soon followed by vomiting, retching, diarrhea, drop in body temperature and culminating in deep torpor or coma. The animals were killed after 4 to 10 days at a time when they exhibited severe signs of uremia. This technique allowed the other constituents in the extracellular fluid to be kept constant while the amounts of urea were varied.

Employing a somewhat similar technique, Johnson and coworkers (58) maintained high blood urea concentrations by intermittent dialysis in three patients suffering from advanced renal failure. Blood concentrations of 181 to 600 mg urea per 100 ml were maintained for periods of 7 to 90 days. When the urea concentration was kept below 300 mg per 100 ml, no untoward effects were noted although this level is about 10 times greater than normal. Concentrations above 300 mg per 100 ml were associated with malaise, vomiting, bleeding tendency, and headache. However, the more severe gastrointestinal, cardiovascular, mental and neurologic changes of uremia were not observed.

Bensinger et al. (59) administered by mouth 40 to 120 g (0.6 to 2.0 g per kg) urea daily in divided doses to eight patients with sickle cell disease for periods of 3 weeks to 9 months. The blood urea concentrations of the patients approximately doubled during the test periods. While the patients were ingesting urea, there was a slight decrease in blood volume, probably resulting from the chronic osmotic diuresis induced by the urea. The life span of the red cells did not change. There was no demonstrable improvement in the patients. The most obvious effects of the urea intake were thirst and diuresis. Two patients were unable to complete the study because of nausea and vomiting.

Long-term studies

Reports of long-term studies of urea were not available to the Select Committee.

Carcinogenesis, teratogenesis, mutagenesis

Urea was injected subcutaneously into 20 Strain A and 10C57 black male mice (60). The dose was progressively increased from 10 to 50 mg (0.5 to 2.5 g per kg body weight). Repeated injections were given over a period of 11 months until a total of 800 mg (40 g per kg body weight) was given. Nineteen mice survived one year and five were killed after 15 months. No induced tumors were observed.

Weekly intraperitoneal injections of 400 mg urea per kg body weight for 13 weeks produced no increase of lung tumors in Strain A mice, a strain sensitive to carcinogenic substances (61).

Immersion of frog eggs in 1.25 percent urea at various times after fertilization produced various embryonic abnormalities, especially of the central nervous system (62). Abnormalities were also produced in chick embryos with relatively small doses of urea (63, 64). Fifty to 900 mg urea dissolved in egg albumin were injected into eggs between the 7th and 20th hour of incubation. Among 132 embryos subjected to this treatment, 78 showed neural, vascular or cardiac abnormalities.

Pregnant rats received a daily dose of 50 g per kg of urea by gastric intubation for an average of 14 days (65). During this period the blood urea levels ranged from 1000 mg per 100 ml one hour after urea administration to 100 mg per 100 ml 12 hours later and just before the next intubation. Within 48 hours, the newborn rats were killed and the kidneys examined. No hypertrophy or other kidney changes were detected nor were any teratogenic effects reported.

Intraruminal administration of 0.44 g urea per kg body weight caused the death of pregnant cows within 4 hours (66). However, when 2 and 1 moles acetic acid per mole urea were injected into the rumen 15 and 180 minutes, respectively, after the urea, the cows survived despite high levels of blood ammonia. When 29 pregnant cows (stage of pregnancy not stated) were treated in this manner only one death resulted. The treatment had no effect on the number of calves born, their birth and weaning weights, and the rebreeding performances of the cows. No abnormalities were reported among the calves.

Urea had no effect on cultured human leukocytes at physiological concentrations (1mM) (67). At a concentration of 50 mM, however, it caused severe chromosome fragmentation and "moderate" cell damage. The authors suggest that these changes may be nonspecific effects of high molarity solutions on cell division.

V. OPINION

Urea is a normal body constituent and is constantly being produced during amino acid and protein metabolism. It is a natural constituent in commonly consumed foods. Several grams per kilogram of body weight can be ingested by nonruminants, including man, without untoward effects. Most of the nitrogen consumed in food is excreted in the form of urea. A 70 kg individual consuming a normal diet will excrete an average of 25 g urea daily. While urea appears to be teratogenic in chick and frog embryos, no teratogenic effects were observed after ingestion of large doses of urea by pregnant rats and cows.

If all urea not used in animal feed and fertilizer were utilized in human food, it would amount to about 5 g per capita daily. However, it is known that the majority of this urea is used for the production of urea-formaldehyde resins and other non-food uses. Therefore, the per capita intake of urea as a direct or indirect food ingredient is much less than 5 g daily.

In the light of the foregoing, the Select Committee concludes that:

> There is no evidence in the available information on urea that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future.

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OCT 27 1978

Date

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007297

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DATA EVALUATION REPORT

STUDY TYPE: Review of Literature on Urea Toxicity

TEST MATERIAL: Urea

Caswell No. 902

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

Project No. 8-1038

MRID No. 407333-08

TITLE OF REPORT: Review of Urea Toxicity

AUTHOR: Dr. Paul Ferguson

CONCLUSIONS AND SUMMARY

Urea is an end product of protein and ammonia metabolism in humans, excreted in the amount of 25-30 g/day (350-420 mg/kg/day). Normal plasma concentrations about 0.26 mg/ml.

Urea is used in animal feed. Therapeutically, in humans, it has been used as an osmotic agent to induce diuresis, and to reduce intraocular and intracranial pressures. It is used in doses of 1-1.5 g/kg for neurosurgical procedures without adverse effects and it is used to debride necrotic and infected tissues. Urea is also used to manufacture plastics and dentifrices.

In 1978, the Select Committee on GRAS Substances of the Life Science Research Office (LSRO) concluded that urea is GRAS. Acute toxicity studies in numerous mammalian species, frogs and pigeons, by oral, s.c. and i.v. routes, indicate toxicity is very low. Urea may cause diuresis in osmolar doses and it is a mild irritant to human skin. Prolonged eye exposure at high concentration caused ocular injury to rabbits.

In several in vitro studies, urea was found to cause chromosomal aberrations in human leukocytes, hamster fibroblasts and lung cells, at concentrations between 50 mM to 8 M, but not at 1 mM physiological levels. It is believed that the chromosomal fragmentation observed was due to a non-specific, hyperosmolarity effect, not a direct effect.

No toxicities have been observed in humans following chronic exposures. In 1-year feeding studies with C57Bl/6 mice and Fischer 344 rats, at doses up to 4.5% of diet, no treatment related tumors have been observed. Weekly i.p. injections of 400 mg/kg for 13 weeks in a sensitive mouse strain caused no induction of lung adenomas.

In a 45 day dog study, doses of 30-40 ml/kg/day of 10% urea resulted in plasma levels of 200-700 mg/100 ml. The only symptoms observed were drowsiness and diuresis with no effect on organ pathology. Toxic clinical manifestations were observed only in nephrectomized dogs in which urea levels of 540-1690 mg/100 ml of extracellular fluid were maintained for 10 days. In human dialysis patients, clinical manifestations are observed with blood urea concentrations above 300 mg/100 ml, but no severe uremia occurs at such levels in the blood.

Intraamniotic injections of osmolar amounts of urea is effective in inducing abortion in monkeys and humans. Pregnant cows which recovered from urea toxicity exhibited no effects on reproductive performance. Pregnant rats receiving doses of 25 g/kg ywice daily by gastric intubation for 14 days produced healthy offspring. Urea has been classified by FDA in category C for therapeutic use because its safety for use during pregnancy has not been established.

STUDY TITLE

Review of Urea Toxicity

DATA REQUIREMENT

Not Applicable

AUTHOR

Dr. Paul Ferguson
(Study is a Literature Search)

STUDY COMPLETED ON

Not Applicable

PERFORMING LABORATORY

Not Applicable

Review of Urea Toxicity

Urea is a neutral, highly water soluble chemical that occurs naturally in humans as an end product of protein and ammonia metabolism. Urea is the primary nitrogenous component of human urine accounting for about 80% by weight of these constituents under conditions of normal protein intake. The absolute amount of urea nitrogen averages about 25-30 grams/day (350-420 mg/kg body weight), all of which is excreted in the urine. The normal plasma concentration of urea is approximately 0.26 mg/ml (2,3).

Urea has been used in animal feed, manufacture of plastics, dentifrices and as an osmotic agent for inducing diuresis and reducing intraocular or intracranial pressure. Intravenous urea doses of 1-1.5 g/kg are optimal for neurosurgical procedures with no adverse effects. Urea has also been used to debride necrotic and infected tissues (1).

From animal studies and human experience, urea toxicity is considered low. This conclusion agrees with that advanced in 1978 by the Select Committee on GRAS Substances of the Life Sciences Research Office (20): "There is no evidence in the available information on urea that demonstrates or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future." The lethal dose (LD_{50}) for an oral exposure in rats was 14,500 mg/kg (5) which would be equivalent to a two pound ingestion of urea by an average size adult human. The acute toxicity of urea has also been evaluated in rabbits, cattle, sheep, dogs, guinea pigs, frogs, pigeons and ponies by oral, subcutaneous and intravenous exposures (20,21). Urea was slightly toxic in these mortality studies. Ruminants (cattle and sheep) are more sensitive to urea than non-ruminants. No serious reactions have been recorded in humans with proper parenteral therapy. Intravenous administration of urea to reduce intracranial pressure may cause headaches (similar to those following lumbar puncture), nausea and vomiting, and occasionally, fainting. Chemical phlebitis and thrombosis near the injection site are rare. At the site of infusion, urea may cause pain and some tissue injury if excessive bleeding occurs (16). Diuresis may be significant.

Urea was considered to be a mild skin irritant when 22 mg urea were applied to human skin intermittently over a 3 day period (5). With parenteral therapy, urea is more irritating to skin than mannitol, another popular osmotic agent.

51

Prolonged eye contact with high concentrations of urea may cause irritation and damage. Exposure of rabbit eyes to a saturated urea solution resulted in loss of epithelium from the cornea after five minutes contact and produced a grayness of the corneal stroma. One hour exposure of a 40% urea solution in rabbit eyes resulted in similar corneal effects with recovery in several weeks. Injection of 0.2 ml of a 10M urea solution (0.12 gms) into the vitreous humor of rabbits caused inflammation, chorioretinitis and retinal degeneration (4).

Several in vitro studies have reported that urea is associated with chromosomal aberrations in human leukocytes, hamster fibroblasts and lung cells (5). All of these studies were conducted with urea concentrations ranging from 50mM - 8M. At physiological levels (1mM) urea causes no chromosome effects. At urea concentrations greater than 50mM the production of chromosome fragmentation is probably a non-specific, hyperosmolarity effect on cell division and not a direct effect of the urea molecule. Sodium phosphate, another normal body fluid constituent also produces chromosome damage at 50mM concentrations (10).

No toxicities from urea have been reported in humans after chronic exposures (6). One year feeding studies in male and female C57Bl/6 mice and Fisher 344 rats reported no evidence of treatment-related cancer at doses up to 4.5% of the diet. Increased incidence of lymphomas in mid-dose female mice, and increased incidence of interstitial cell adenomas of the testes in high dose male rats were not considered biologically significant (7).

Earlier studies also indicated no evidence of urea tumorigenicity. Doses of 0.5-2.5 g/kg were injected subcutaneously over a period of 11 months. No tumors were evident after 15 months. Weekly intraperitoneal injections of 400 mg/kg for 13 weeks produced no lung adenomas in sensitive Strain A mice (20).

Urea produced no severe toxicity in dogs injected subcutaneously every 8 hours with 30-40 ml/kg of 10% urea solution for 45 days. With plasma levels ranging from 200-700 mg/100 ml (10-30 fold above normal) only the expected symptoms of drowsiness and diuresis were evident. Necropsy indicated no adverse organ pathology. Therefore, from this study urea was not considered an important uremic toxin (13). However, in studies of nephrectomized dogs in which urea levels of 540-1690 mg/100 ml of extracellular fluid were maintained for 10 days, uremic signs such as vomiting, diarrhea, reduced body temperature and coma were evident. At blood urea concentrations above 300 mg/100 ml human dialysis patients exhibited malaise, vomiting, bleeding tendency and headache, but severe forms of uremia were not observed (20).

Due to its effectiveness as an osmotic agent, urea has been evaluated in monkeys and humans for ability to induce abortion (11,12,17,18). Intra-amniotic injection of 80 grams "Ureaphil"/210 ml 5% dextrose was effective in inducing abortion at 14 weeks without adverse effects to the mother. The mode of action is similar to the hyperosmolar effect of large doses of hypertonic saline and dextrose where a highly localized, hyperosmolar solute passes from the amniotic fluid into the fetus causing death. Such high intrauterine exposures would not occur from environmental exposure to urea. In fact, pregnant cows which recovered from urea toxicity exhibited no effects on reproductive performance nor were the calves affected. Cows treated acutely (.44 gms/kg) and kept under regular management for 12 months showed no effect on number of calves born, birth weight, weaning weight of calves or rebreeding performance of cows (14). In a limited study, pregnant Wistar rats receiving a twice - daily dose of 25 g/kg by gastric intubation for 14 days produced [illegible]hy offspring with no reported evidence of teratogenic effects even though maternal blood urea levels reached 100-1000 mg/100 ml (20). Immersion of frog eggs in 1.25% urea and injection of chick embryos with 50-900 mg urea have produced some evidence of neural, vascular or cardiac abnormalities (20). Urea is currently classified in category C for therapeutic use ("Safety for use during pregnancy has not been established" (16)).

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SStolzenberg 6/12/89
J.M.J. 6-13-89

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral toxicity

Caswell No. 902

GUIDELINE: 81-1

HED Project No. 8-1038

TEST MATERIAL: Unocal Plus

MRID No. 407333-04

SYNONYMS: Urea, Enfrost

STUDY NUMBER(S): 480-2777

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TESTING FACILITY: American Biogenics Corp
Decatur, IL 62526

TITLE OF REPORT: Acute Oral Toxicity Study in Rats with Unocal Plus

AUTHOR(S): Sandra Smith

REPORT ISSUED: Sept. 24, 1986

CONCLUSIONS AND RECOMMENDATIONS

Classification: Core Supplementary

The purity of urea used and the percentage of urea in the test substance were not indicated. This study may be upgraded when this information is received. The following values should be assigned if upgraded.

LD50 > 5 g/kg (Limit dose test). Tox. Category IV

A. MATERIALS

1. Test Compound: Unocal Plus. Description: A liquid. Purity, contaminants and inert material composition are not listed. Density of substance was determined to be 1113.1 mg/ml

2. Test animals: "Young adult" Sprague-Dawley rats from Charles River in Portage, MI. Body weight on day 0 of the study was 262 ± 18 g for males, 201 ± 5 g for the females.

B. PROCEDURES

Five of each sex received 5 g/kg, (limit dose test) by oral gavage. Body weights were obtained on days 0, 7 and 14. Necropsies were performed under supervision of a pathologist. Animals were observed for clinical signs throughout the study.

C. COMPLIANCE

- A signed Quality Assurance statement was included.

D. RESULTS

No effect of treatment was observed.

E. CONCLUSION:

Classification: Core Supplementary

The purity of urea and composition of urea in the test substance were not indicated. This classification may be upgraded when this information is supplied. The following values are recommended if the study is upgraded.

LD_{50} > 5 g/kg Tox. Category IV

007297

Reviewed by: Sidney Stolzenberg, Ph.D. SPStolzenberg 6/12/89
Section I, TOX Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Michael Ioannou, Ph.D. JMI 6-13-89
Section I, TOX Branch II, HFAS/HED (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity

Caswell No. 902

GUIDELINE: 81-2

HED Project No. 8-1038

TEST MATERIAL: Unocal Plus

MRID No. 407333-05

SYNONYMS: Urea, Enfrost

STUDY NUMBER(S): 480-2778

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TESTING FACILITY: American Biogenics Corp.
Decatur, IL. 62526

TITLE OF REPORT: Acute Dermal Toxicity Study in Rabbits with Unocal Plus.

AUTHOR(S): Sandra Smith

REPORT ISSUED: Sept. 23, 1986

CONCLUSIONS AND RECOMMENDATIONS

Core Classification: Supplementary

The purity of urea and composition of urea in the test substance were not indicated. The classification may be upgraded when this information is supplied. The following values are recommended if this study is upgraded.

LD50 > 2 g/kg Tox. Category III

58

A. MATERIALS

1. Test compound: Unocal Plus. Description: Liquid, Lot No. AO-4201, density determined to be 1113.1 mg/ml

2. Test animals: New Zealand White young rabbits (age not specified), males and females, weighing 2.5 to 3.2 kg at the start of the study.

3. Dosage: Neat substance, 2 g/kg body weight, applied to shared skin on about 10% of body surface with about 22.8 mg/cm^2 of body surface.

B. STUDY DESIGN AND METHODS

Five of each sex were used. Test substance was held in place with plastic wrapper impervious to compound. The compound was removed after 24 hours of skin contract. Observation period was for 14 days with body weights obtained on days 0, 7 and 14. Animals were subjected to gross pathology on day 14.

C. COMPLIANCE

- A signed statement of Quality Assurance was provided.

D. RESULTS

No effect on clinical signs, observed each day, no effect on weight gain; all animals survived to day 14. Erythema was present on day 2 in 3 males and all 5 females. No abnormalities were observed at necropsy.

E. CONCLUSION

Core Classification: Supplementary

The purity of urea and composition of urea in the test substance were not indicated. The classification may be upgraded when this information is supplied. The following values are recommended if this study is upgraded.

LD50 > 2 g/kg Tox. Category III

007297

Reviewed by: Sidney Stolzenberg, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Michael Ioannou, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C)

S J Stolzenberg 6/12/89
M.I. 6/12/89

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation

Caswell No. 902

GUIDELINE: 81-3

HED Project No. 8-1038

TEST MATERIAL: Unocal Plus

MRID No. 407333-01

SYNONYMS: Urea, Enfrost

Lab. STUDY NUMBER(S): I-7090.112

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TESTING FACILITY: Microbiological Associates
Bethesda, MD 20816

TITLE OF REPORT: Acute Inhalation Toxicity Study of Unocal Plus in the Rat.

AUTHOR(S): R.M. David, PhD., DABT

REPORT ISSUED: May 11, 1988

CONCLUSIONS AND RECOMMENDATIONS

The substance tested is considered to be Enfrost, previously called Unocal Plus. This product has a urea content of 42.5%.

Inhalation LC_{50} > 4.8 mg/L/4 hours. Tox. Category III

Core Classification: Minimum

60

A. MATERIALS

1. Test compound: Unocal Plus. Description: Yellow liquid. Lot: Reference No. AP 4186, test article no. T07090A. Purity: 42.5% urea. Contaminants and inert materials were not listed.

2. Test animals: Rats, Fischer 344, 9 week old when treated; body weights were 193-217 g for males, 138-160 g for females, on treatment day.

B. STUDY DESIGN AND METHOD

Five males and 5 females were exposed to a dose of 4.8 mg/L nominal concentration, the highest attainable concentration of the aerosolized test substance. A Collision Nebulizer was used to generate the aerosol. There was no mention of temperature or solvent used to assist in vaporization of the test substance. Nose only exposure units were used. Five animals were restrained per unit exposure. Air flow was approximately 5L/min, which was periodically monitored. Sampling of test atmosphere was done from a sampling port situated on the side of the exposure module (apparently at only one location). Samples were analyzed for gravimetric and particle size determinations. Five separate determinations were made and a time-weighted average was calculated for total aerosol concentration. Particle size was determined by using a Mercer 7 stage cascade impactor with cutoff stages for 7 different particle sizes ranging from 0.5 to 10 micron. Oxygen determinations were made in the chamber and set at 20% throughout the exposure period. Samples from the neat material and from the atmosphere were analyzed for urea. A "sham exposed" control group received the same restraint in the exposure chamber as the treated rats with the same air flow rate. Exposure period was for 4 hours.

Observations included clinical signs at 1 and 3 hours post-exposure, then once daily thereafter to day 14. Complete necropsies were performed on day 15 when organs with lesions were preserved. Body weights were obtained at pre-dose, then weekly.

C. COMPLIANCE

- A signed statement of compliance with EPA's GLP was provided.

61

D. Results

The highest concentration of Unocal Plus actually attained in the chamber, indicated in the text, was 3.5 mg/L. Analytical concentration of urea in test atmosphere was 70 %, compared to 48.5% urea in the neat material. No table of results for these determinations were found. Average for mass median diameter was 2.75 microns, based on 2 determinations in which the actual results came to 2,4 and 3.1 microns.

Respirable concentration (< 15 microns), based on size distribution, was 95-99% of the 4.8 mg/L.

Animal Observations.

Mortality: None

Cinical signs: Red color secretions from the nose, observed on at 1 hour after exposure, was not seen at 3 hours or after. This was the only compound related effect.

Body weights: No compound related effect.

Gross pathology: No gross lesions were observed.

The LC_{50} is considered to be > 4.8 mg/L/4 hours

007297

Reviewed by: Sidney Stolzenberg, Ph.D. S J Stolzenberg 6/12/89
Section I, TOX Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Michael Ioannou, Ph.D. J.M.I. 6-13-89
Section I, TOX Branch II, HFAS/HED (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irritation

Caswell No. 902

GUIDELINE: 81-4

HED Project No. 8-1038

TEST MATERIAL: Unocal Plus

MRID No. 407333-02

SYNONYMS: Urea, Enfrost

STUDY NUMBER(S): 480-2780

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TESTING FACILITY: American Biogenics Corp
Decatur, IL. 62526

TITLE OF REPORT: Primary Eye Irritation Study in Rabbits with Unocal Plus.

AUTHOR(S): S. H. Smith

REPORT ISSUED: June 25, 1988

CONCLUSIONS (Summary)

Classification: Core Supplementary

The purity of urea and content of urea in the test substance were not stated in the report. The following P.I.S. values and Toxicity Category are recommended if the study is upgraded.

	Unwashed	Washed
P.I.S. at 1 Hour:	3.2	9.0
24 Hours:	0.0	4.0
48 Hours:	0.0	0.0

Tox Category IV for primary eye irritation; minimallly irritating, clearing within 24 hours in unwashed eye, within 48 hours in washed eye.

63

A. MATERIALS

1. Test compound: Unocal Plus. Description: Liquid, pH 6.0 approximately. Lot No. A04201. Contaminants are not listed; stored at ambient laboratory conditions.

2. Test animals: Rabbits, New Zealand White, obtained from Clerco Research Farm in Cincinnati, OH. "Young Adult" animals were used but their age or body weight were not indicated.

B. STUDY

Dosage: 0.1 ml, without dilution.

Route: Instilled into the everted lower lid of the right eye of each animal. The eyelids were held together for about 1 second.

C. METHODS: Six males on test had no washing of eye after dosing. Three females had the treated eye washed with warm tap water for one minute at a rate of approximately one liter/minute about 30 seconds after instillation of test substance into the eye. The treated eye was examined for irritation and lesions at 1, 24, 48 and 72 hours. Fluorescein, 2% solution, was used as an aid for evaluation at all time periods, except for the one hour period. Eye irritation ratings were determined according to the method of Kay and Calandra (J. Soc. Cosmetic Chemists 13: 281-289, 1962), which uses a Draize scoring system.

D. COMPLIANCE:

- A signed Quality Assurance Statement was provided.

E. RESULTS

The following are mean values for the six unwashed and three washed treated eyes, calculated from the individual animal results of Tables 2 and 3 in the submitted report.

	1 Hour		24 Hour		48 Hour		72 Hour	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
I. Cornea								
A. Density	0	0	0	0	0	0	0	0
B. Area	0	0	0	0	0	0	0	0
II. Iris	0.2	0.3	0	0.3	0	0	0	0
III. Conjunctivae								
A. Redness	0.8	1.0	0	1.0	0	0	0	0
B. Chemosis	0.3	2.0	0	1.0	0	0	0	0
C. Discharge	0	0.7	0	0	0	0	0	0

F.CONCLUSION:

Classification: Core Supplementary

There is no information on purity of urea or ccontent of urea in the test substance. The following P.I.S. values and Toxicity categories are recommended if this study is upgraded.

	Unwashed	Washed
P.I.S. at 1 hours:	3.2	9.0
24 hours:	0.0	4.0
48 hours:	0.0	0.0
72 hours:	0.0	0.0

Tox Category IV for primary eye irritation (minimally irritating, clearing within 24 hours for unwashed eye 48 hours for washed eye).

007297

Reviewed by: Sidney Stolzenberg, Ph.D. S.J. Stolzenberg 6/12/89
Section I, TOX Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Michael Ioannou, Ph.D. J.M.I. 6-13-89
Section I, TOX Branch II, HFAS/HED (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Primary Dermal Irritation

Caswell No. 902

GUIDELINE: 81-5

HED Project No. 8-1038

TEST MATERIAL: Unocal Plus

MRID No. 407333-06

SYNONYMS: Urea, Enfrost

STUDY NUMBER(S): 480-2779

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TESTING FACILITY: American Biogenics Corp
Decatur, IL 62526

TITLE OF REPORT: Primary Dermal Irritation in Rabbits with Unocal Plus

AUTHOR(S): Sandra Smith

REPORT ISSUED: Sept. 12, 1986

CONCLUSIONS AND RECOMMENDATIONS

Classification: Core Supplementary

There is no information on purity of urea and composition of urea in the test substance. The study should be upgraded if this information is supplied and the following values assigned.

Mean P.I.S Scores: 0.2 at 30-60 minutes
0 at 72 hours

Tox. Category IV. Minimally irritating

A. MATERIALS

1. Test compound: Unocal Plus. A liquid, Lot # A04201, pH 6.0. Purity was not indicated.

2. Test animals: New Zealand Albino rabbits "young adult", weights are not indicated. Obtained from Clerco Research Farm, Cincinnati, OH.

B. STUDY PROCEDURE

Six male rabbits were shaved, at 4 different sites per rabbit, on each side of the thoracic region of the spinal column. Two shaved sites, both on the right side were abraded, the other 2 sites were left intact. 0.5 ml of neat test substance was applied by means of a 2.5 cm^2 gauze patch, to each of the 4 prepared test sites on all 6 rabbits. After 4 hours of exposure, test substance was removed from each test site and evaluated for erythema, edema and other possible lesions at 30-60 minutes, 24, 48 and 72 hours.

C. COMPLIANCE

- A signed statement of Quality Assurance was submitted.

D. Results

No mortalities or clinical signs were observed.

PIS Scores:

Time	Mean Score	
30-60 min	0.1	Minimally irritating
24 hours	0.1	
48 hours	0	
72 hours	0	

E. CONCLUSION

Classification: Core Supplementary

The purity of urea and composition of urea in the test substance were not stated in the report. The study may be upgraded when the information is supplied.

Tox. Category: IV

This value should be assigned if the study is upgraded.

Reviewed by: Sidney Stolzenberg, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C) S Stolzenberg 6/12/89
Secondary Reviewer: Michael Ioannou, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C) J M.I 6-13-89

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization

GUIDELINE: 81-6

TEST MATERIAL: Unocal Plus

SYNONYMS: Urea, Enfrost

STUDY NUMBER(S): 480-2781

Caswell No. 902

HED Project No. 8-1038

MRID No. 407333-03

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TESTING FACILITY: American Biogenics Corp
Decatur, IL 62526

TITLE OF REPORT: Dermal Sensitization Study in Guinea Pigs with Unocal Plus

AUTHOR(S): Sandra Smith

REPORT ISSUED: Sept. 24, 1986

CONCLUSIONS AND RECOMMENDATIONS

No skin sensitization was seen.

Core Classification: Supplementary

The purity of urea and percentage of urea in the test substance was not indicated. The classification may be upgraded when this information is supplied.

A. MATERIALS

1. Test Compound: Unocal Plus. Description: A liquid. Lot # AO-420. Purity of urea and percentage of urea in the test substance were not listed.

2. Test animals: Guinea pigs, Hartley strain, young adults; age and weight were not specified. Obtained from Harlan Sprague Dawley, Inc., Indianapolis, IN.

B. PROCEDURES

Range Finding Study: Two guinea pigs received 0.5 ml of varied concentrations, diluted by suspension in deionized water. There was a 6 hour exposure duration for each application. Two concentrations were evaluated per animal, using suspensions of 3, 10, 30 and 100%.

Main Study: Consisted of 2 groups of 10 animals. In the test group, 9 induction applications of 0.5 ml undiluted test substance was applied to the shaved skin on the back of flank, 3 times per week (3 weeks); 6 hour exposure period for each application of 0.5 ml undiluted test material was placed on the shaved skin. The second group of test animals was a "naive" control group. Animals in naive control group received no induction applications but received only the challenge dose of 0.5 ml for a 6 hour exposure period. A 4X4 cm Webril patch was used to apply the test substance for each induction and challenge application.

After removal of the patch, the sites on each animal were examined for erythema, edema and other lesions 24 hours after each application. Evaluation by a grade of 0 to 4 according to Draize scoring systems for erythema and for edema were used 24 and 48 hours after each application.

There was no concomitant positive control with this study. Instead, an historic positive control, performed in November, 1985, was used for comparison. For this positive control, 10 guinea pigs received a 0.1% suspension of dinitrochlorobenzene in acetone for the induction and challenge phases of the study.

C. COMPLIANCE

- A signed Quality Assurance statement was provided

C. Results

Range Finding Study: No erythema or edema was observed. Therefore, the test compound was tested in undiluted form in the main study.

Main Study: In the induction phase at 24 hours, very slight erythema was observed, but no edema, in 2 of 10 animals after the first skin application. Subsequently, very slight erythema in only 1 animal of 10 in the group was seen after induction #6, 7, 8, and 9. Each consisted of a score of 1 for erythema.

No skin sensitization reaction was observed in any animals in test group or in the naive control group.

Classification: Supplementary

The purity of urea and the percent of urea in the test substance were not indicated.

A

ATTACHMENT 2

Petition for use of Urea on crops as a frost protection agent. Request to waive certain toxicity data requirements. PP# 8F3662. (Memorandum: D. Ritter, February 24, 1989).

An electronic version of this document is not available. See the hard copy file.

71

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

007048

FEB 24 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

Subject: PP # 8F3662 - Petition for use of Urea on crops as a frost protection agent. Request to waive certain toxicity data requirements.

TO: Eugene Wilson, PM # 23
Herbicide/Fungicide Branch
Registration Division TS-767C

Caswell #: 902.
Potomac #: P594.
TOX Proj. #: 8-1038.

THRU: Yannakis M. Ioannou, Ph.D, Acting Section Head
Rev. Sec. # I/HFASB
Health Effects Division TS-769C

J.M. Ioannou 2-22-89

THRU: Marcia Van Gemert, Ph.D., Acting Chief
HFASB
Health Effects Division TS-769C

K. Clark Smartzel for 2/24/89

FROM: D. Ritter, Toxicologist
Rev. Sec. # I/HFASB
Health Effects Division TS-769C

DR 2-22-89

Registrant: Unocal Chemicals Division, Unocal Corporation.

Registrant is requesting waiver of certain toxicity data requirements for this Significant New Use product containing Urea for prevention of frost in racs.

The bases for this request as cited by the Registrant are as follows:

1. Urea has GRAS status as a direct food additive under 21 CFR 184.1923.

2. Urea is exempt from the requirement of a tolerance under 40 CFR 180.1001(c) as an inert ingredient in formulations applied to growing crops or to crops after harvest.

72

3. The amount to be used is similar to that permitted for the inert ingredient use.

4. Urea is a normal constituent of animal tissues and body fluids. Man excretes about 25 grams per day in the urine.

5. Urea is a naturally occurring crop/plant constituent.

The list of studies to be waived are:

1. Ninety Day Feeding in rodent and non-rodent (82-1).
2. 21 Day Dermal (82-2).
3. 90 Day Dermal (82-3).
4. 90 Day Inhalation (82-4).
5. Chronic Feeding in rodent and non-rodent (83-1).
6. Oncogenicity in rat and mouse (83-2)
7. Teratogenicity in 2 species (83-3).
8. Reproduction - 2 generation (83-4)
9. Mutagenicity battery (84-2 thru 84-4)
10. General Metabolism (85-1)

Recommendation:

Toxicology Branch has no objection to waiving the cited data requirements based on the reasons noted above.

ATTACHMENT 3

Urea (Enfrost); Reclassification of Six Acute Toxicity Studies from Supplementary to Guideline (Memorandum: R. Landolt, June 17, 1991).

An electronic version of this document is not available. See the hard copy file.

CASWELL FILE

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

JUN 19 1991

008416

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Urea (Enfrost)

HED Project No.: 1-1307
TOX Chem No.: 902

FROM: Ray Landolt 6/17/91
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

TO: Cynthia Giles-Parker, PM 22
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: Mike Ioannou, Section Head J M Ioannou 6/17/91
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)
and
Marcia van Gemert, Chief Marcia van Gemert 6/18/91
Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Unocal Chemicals Div., letter of April 15, 1991.
EPA Reg. No. 612-A, (Enfrost) containing 42.9% Urea.
Pesticide Petition 8F3662.

Action Requested: In response to the Toxicology review (DER 007297) of June 20, 1989 by Sidney Stolzenberg, the registrant has

1. Provided the concentration of the test material administered in those acute studies found deficient in this review and

2. Has addressed our concern for an exemption of an inert ingredient listed in this formulation of Enfrost.

Recommendation: 1. The acute oral and dermal toxicity studies, the primary dermal and eye irritation studies and the dermal sensitization study were conducted with 42.9% Enfrost. These studies may be upgraded from Supplementary to Guideline and support the registration of Enfrost.

2. The registrant, with the submission of Pesticide Petition 9F3792 (August 15, 1989) requested this inert ingredient to be listed in 40CFR 180.1001.

75

Consideration given this request:

The following acute studies were classified Supplementary in the Toxiciology review (DER 007279) of June 20, 1989 for the lack of information on the purity and composition of the test material.

These studies were conducted with a 39.5% (analytical analysis) concentration of urea and may be upgraded to Guideline.

The following studies satisify the guideline data requirement (158.135) and support the signal word Caution and Toxicity Category III precautionary labeling for this formulation of urea.

	Study	MRID No.	Toxicity Category
(81-1)	Acute oral toxicity	407333-04	IV
(81-2)	Acute dermal toxicity	407333-05	III
(81-3)	Acute inhalation	407333-01	III
(81-4)	Primary eye irritation	407333-02	IV
(81-5)	Primary dermal irritation	407333-06	IV
(81-6)	Dermal sensitization	407333-03	Negative

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Metabolism Species: rat	Urea tech		Data requirement waived		007048
Mutagenic·Ames Species: bacteria	Urea tech		Data requirement waived		007048
Mutagenic·chromosome aberr. Species:	Urea tech		Data requirement waived		007048
Mutagenic·DNA repair test Species:	Urea tech		Data requirement waived		007048
Acute inhalation LC50 Species: rat Microbiological Associates 5/11/88	Entrost 42.5% Urea Lot #AP4186	407333-01	LC50 > 4.8 mg/l/4 hr	3	Minimum 007297
Primary eye irritation Species: rabbit American Biogenics Corp. 6/25/88	Entrost Purity not indicated	407333-02	PIS at 1 hr: 3.2; at 24 hr: 0.0. Minimal irritation, clearing within 24 hrs in unwashed eye.	4	Supplementary 007297
Dermal sensitization Species: guinea pig American Biogenics Corp. 9/24/86	Entrost ~~purity not indicated~~	407333-03	No skin sensitization was seen		Supplementary 007297
Acute oral LD50 Species: rat American Biogenics Corp. 9/24/86	Entrost purity not indicated	407333-04	LD50 > 5 g/kg (limit dose test)	4	Supplementary 007297
Acute Dermal LD50 Species: rat American Biogenics Corp. 9/23/86	Entrost ~~purity not indicated~~	407333-05	LD50 > 2 g/kg	3	Supplementary 007297
Primary dermal irritation Species: rabbit American Biogenics Corp.	Entrost purity not indicated	407333-06	PIOS at 30 - 60 min : 0.2; at 72 hrs: 0	4	Supplementary 007297

77

ATTACHMENT 4

Data waiver request for urea as an active ingredient for use as a frost protectant (Memorandum: J. Stewart, April 17, 2001).

An electronic version of this document is not available. See the hard copy file.

78

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

(4-17-2001)

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Data Waiver Request for Urea as an active ingredient for use as a frost protectant.

To: Michelle Centra, Ph.D.
Reregistration Branch 3
HED (7509C)

From: Joycelyn E. Stewart, Ph.D., Chair,
Toxicology Science Advisory Council
HED (7509C)

The Toxicology Science Advisory Council met on 3/30/ 2001 to consider a request to affirm the data waivers granted for the use of urea ($CO(NH_2)$ Cas No 57-136; as a frost protectant on food crops. Present were: Mike Ioannou, Joycelyn Stewart (Chairman) Ed Br[illegible]; Roger Gardner, John Whalan, John Doherty, Bill Dykstra.

Data waivers were granted in 1989 for the following studies (see Memoranda:Ritter to Wilson dated 2/23/1989 and Stolzenberg to Rossi dated 6/13/1989).

1. 90 Day Feeding Study in rodents	870.3100
2. 90 Day Feeding Study in nonrodents	870.3150
3. 21 Day Dermal Toxicity Study	870.3200
4. 90 Dermal Toxicity Study	870.3250
5. 90 Day Inhalation Toxocity Study	870.3465
6. Chronic Feeding Studies in rodents and nonrodents	870.4100
7. Carcinogenicity Studies in 2 mammalian species	870.4200
8. Developmental Toxicity Studies in rodents and nonrodents	870.3700
9. Multigeneration Reproduction Study in rodents	870.3800
10Battery of Mutagenicity Studies	870.5100; 870.5300; 870.5385 870.5375 and 870.5395
11.General Metabolism Study	870.7485

79

The bases for these waivers were as follows:

Urea was GRAS as a direct food additive under 21 CFR 184.1923

The amount to be used was similar to that permitted for the inert ingredient use in pesticide formulations and the level commonly used in fertilizer applications.

Urea was exempt from the requirements of a tolerance under 40 CFR 180.1001 (c) as an inert ingredient in formulations applied to growing crops or to crops after harvest.

Urea is a natural plant/crop constituent. Up to 4.5% of the nitrogen in oats. Up to 15% of total nitrogen in young plants and 5% in mature plants is non-protein nitrogen, much of which is in the form of urea.

Urea is a normal constituent of animal tissues and body fluids. Man excretes abaouat 25 grams per day in the urine.

Discussion:

The TOX-SAC examined the 1978 Monograph on Urea by the FDA Select Committee on GRAS Substances of the Life Sciences Research Office (LSRO). Federation American Society of Experimental Biology (FASEB)as well as the HED One liners and the 21 CFR Citation 184.1923 which affirms urea as GRAS as a direct human food ingredient. It was noted that the FDA GRAS affirmation was without limitations other than current good manufacturing practice. There are no prior sanctions for this chemical.

Conclusion

Based on the information presented to the TOX-SAC , the Council voted unanimously to re-affirm the data waivers, and to recommend that no further studies be required.

This document is filed under T:HED\SAC\TOXSAC\MINUTES\TOXMIN16

ATTACHMENT 5

Urea (Enfrost, 42.9% a.i.): Reevaluation of mammalian acute toxicity studies (OPPTS Test Guidelines 870.1100, 870.1200, 870.1300, 870.2400, 870.2500, and 870.2600) submitted by Unocal Corporation in Support of Registration. PC Code: 085702. DP Barcode: D277687. (Memorandum: M. Centra, August 28, 2001).

An electronic version of this document is available and stored under the following Toxicology Record Number (TXR#): 0050171.

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

TXR Number: 0050171

MEMORANDUM

DATE: March 10, 2002

SUBJECT: ***ENFROST* (Urea, End-Use Formulation):** Re-evaluation of mammalian acute toxicity studies (OPPTS Test Guidelines 870.1100, 870.1200, 870.1300, 870.2400, 870.2500, and 870.2600) submitted by Unocal Corporation in Support of Registration.

PC Code: 085702 PRAT Case No.: 819300 Case No.: 4096
DP Barcode: D277687 Submission No.: S596788

FROM: Michelle M. Centra, Pharmacologist Michelle M. Centra
Reregistration Branch III
Health Effects Division (7509C)

THRU: Catherine Eiden, Branch Senior Scientist for
Reregistration Branch III
Health Effects Division (7509C)

TO: Joseph Nevola, Chemical Review Manager
Robert McNally, Branch Chief
Special Review Branch
Special Review and Reregistration Division (7508W)

Introduction: In 1995, the Agency established a permanent exemption from the requirement of a tolerance for residues of the frost protectant pesticide, Urea, in or on various agricultural commodities. However, since these permanent tolerance exemptions were issued prior to passage of the Food Quality Protection Act (1996), a revised hazard characterization was required for this pesticide. The Health Effects Division (HED) has reassessed the available toxicity data for Urea, including the acute toxicity studies submitted by the Unocal Corporation in support of registration. The submitted studies include: acute oral, dermal and inhalation toxicity studies, primary eye and dermal irritation studies, and a skin sensitization study. This

memorandum contains an appended executive summary for each of the acute toxicity study reviews. The original data evaluation records (DERs) are also attached.

Action Requested: Re-evaluate the mammalian acute toxicity studies submitted by Unocal Corporation in Support of the Registration of Enfrost (a urea containing end-use formulation) in accordance with the OPPTS Harmonized Test Guidelines 870.1100, 870.1200, 870.1300, 870.2400, 870.2500, and 870.2600.

Agency Conclusion: Reregistration Branch III has evaluated the Enfrost acute toxicity studies and determined that the following does fulfill the testing guideline requirements: acute oral toxicity in rats (OPPTS 870.1100), acute dermal toxicity in rabbits (OPPTS 870.1200), acute inhalation toxicity in rats (OPPTS 870.1300), primary eye irritation in rabbits (OPPTS 870.2400), primary dermal irritation in rabbits (OPPTS 870.2500), and skin sensitization in guinea pigs (OPPTS 870.2600). Each study is classified as **Acceptable/Guideline** and is adequate for regulatory purposes.

1. STUDY TYPE: Acute Oral Toxicity - Rat [OPPTS 870.1100 (§81-1)]

CITATION: Smith, Sandra (1986): Acute Oral Toxicity Study with Rats with Unocal Plus. American Biogenics Corporation, 1800 East Pershing Road, Decatur, IL 62526. Study Number ID: 480-2777, September 24, 1986. MRID 40733304. Unpublished.

EXECUTIVE SUMMARY: In an acute oral (limit dose) toxicity study (MRID 40733304) five male and five female fasted, young adult, Sprague-Dawley rats received a single oral gavage dose of 5000 mg/kg Enfrost (urea, 42.9% a.i.). Body weights were measured prior to test material administration (day 0), on day 7, and prior to sacrifice on day 14. Animals were observed at least once daily for mortality and abnormal clinical signs throughout the study and subjected to gross pathology examination on day 14.

All animals survived until study termination and gained an appropriate amount of weight throughout the observation period. Clinical signs of toxicity were not observed during the study and necropsy evaluation of all animals revealed no abnormal findings.

The acute oral LD_{50} value for male and female rats is greater than 5000 mg/kg (limit dose). Enfrost (urea, 42.9% a.i.) is assigned to TOXICITY CATEGORY IV based on the oral LD_{50} value established in this study.

This acute oral study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for an acute oral (limit dose) toxicity study [870.1100 (§81-1)] in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and [No] Data Confidentiality statements were provided.

2. STUDY TYPE: Acute Dermal Toxicity - Rabbit [OPPTS 870.1200 (§81-2)]

CITATION: Smith, Sandra (1986): Acute Dermal Toxicity Study in Rabbits with Unocal Plus. American Biogenics Corporation, 1800 East Pershing Road, Decatur, IL 62526. Study Number ID: 480-2778, September 23, 1986. MRID 40733305. Unpublished.

EXECUTIVE SUMMARY: In an acute (limit dose) dermal toxicity study (MRID 40733305), New Zealand white rabbits (5/sex) were dermally exposed to a single dose of 2000 mg/kg Enfrost (urea, 42.9% a.i.) for 24 hours. Body weights were measured prior to test article application (study day 0), on study day 7, and prior to sacrifice (study day 14). The animals were observed for clinical signs of toxicity throughout the study and subjected to gross pathology examination on day 14.

At 2000 mg/kg, treatment-related erythema (skin reddening) occurred in 8/10 rabbits (3/5 males and 5/5 females) on day 2 of the study. Dermal irritation was resolved in all of the affected animals by study day 3. All animals survived the 14 day observation period and gained an

appropriate amount of body weight throughout the study. There were no clinical signs of toxicity noted and necropsy revealed no gross pathologies in any animal treated with 2000 mg/kg test compound.

The dermal LD_{50} value for male and female rabbits was greater than 2000 mg/kg. Enfrost (urea, 42.9% a.i.) is assigned to TOXICITY CATEGORY III based upon the dermal (limit dose) LD_{50} value established in this study.

This acute dermal study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for an acute dermal toxicity study [870.1200 (§81-2)] in rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Statement of Compliance and [No] Data Confidentiality statements were provided.

3. STUDY TYPE: Acute Inhalation Toxicity - Rat [OPPTS 870.1300 (§81-3)]

CITATION: Smith, Sandra (1986): Acute Inhalation Toxicity Study with Rats with Unocal Plus. American Biogenics Corporation, 1800 East Pershing Road, Decatur, IL 62526. Laboratory ID Number: Study I-7090.112, May 11, 1988. MRID 40733301. Unpublished.

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 40733301), Fischer 344 rats (5/sex) were exposed "nose only" to the highest attainable nominal concentration (4.8 mg/L) of Enfrost (urea, 42.5% a.i.) for 4 hours. A sham control group (used to control for the biological effects of restraint) consisting of 5 male and 5 female rats was exposed to air alone for 4 hours. Flow rate was set at 0.5 L/min and the Mass Median Aerodynamic Diameter (MMAD) for the 4 hour Enfrost exposure ranged from 2.4 to 3.1 microns (average: 2.75 microns). Body weights were measured prior to treatment and once weekly thereafter. The animals were observed for mortality and clinical signs at 1 and 3 hours following exposure, then once daily thereafter for the duration of the study period (14 days). Complete necropsies were performed on all animals on day 15 of the study.

Eye tearing was observed in both treated (5/5 males, 5/5 females) and sham control (5/5 males, 5/5 females) animals during exposure and at the one and three hour post-exposure evaluation intervals. All animals (including the sham controls) presented with hunched posture, ruffled and soiled fur (stained brown) and red colored secretions from the eyes (tearing) at one hour post-treatment. A red colored secretion from the nose was also observed in treated rats (5/5 males, 5/5 females) one hour after exposure, however, nasal secretions were no longer evident by 3 hours. Rats appeared normal by 24 hours post-treatment and no unusual behavior or appearance was observed for the remainder of the test period (14 days). There were no mortalities and no significant differences in body weight/body weight gain between treated and sham control groups. No gross lesions were observed at necropsy.

Based on these results, the LC_{50} for Unocal Plus (Urea, 45.3% a.i.) is greater than 4.8 mg/L.

This acute inhalation study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for an acute inhalation toxicity study [870.1300 (§81-3)] in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Statement of Compliance and [No] Data Confidentiality statements were provided.

4. STUDY TYPE: Primary Eye Irritation - Rabbit [OPPTS 870.2400 (§81-4)]

CITATION: Smith, Sandra (1986): Primary Eye Irritation Study in Rabbits with Unocal Plus. American Biogenics Corporation, 1800 East Pershing Road, Decatur, IL 62526. Study Number ID: 480-2780, September 12, 1986. MRID 40733302. Unpublished.

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 40733302), New Zealand white rabbits (six males and three females) received 0.1 ml of Enfrost (urea, 42.9% a.i., undiluted) in the lower lid of the right eye. The treated eyes of three female rabbits were rinsed with warm tap water for 30 seconds following instillation of the test substance while the treated eyes of six male rabbits were left unwashed (the left eye remained untreated and served as the control). Each treated eye was evaluated for ocular irritation and lesions using fluorescein stain at 24, 48, and 72 hours (the 1 hour evaluation time period was performed without fluorescein stain). Eye irritation ratings were determined according to the method of Kay and Calandra (J. Soc. Cosmetic Chemists 13: 281-289, 1962), which uses a Draize scoring system. Animals were observed for mortality and clinical signs at least once daily during the study period (72 hours).

No mortalities occurred and no abnormal clinical signs were observed during the study. At the1 hour evaluation interval, animals in the unwashed eye group exhibited redness (5/6 males, grade 1), chemosis (2/6 males, grade1), and iritis (1/6 males, grade 1). All ocular irritation occurring in the unwashed eyes of male rabbits was resolved by 24 hours. Redness (3/3 females, grade 1 at both the 1 and 24 hour evaluation intervals), chemosis (3/3 females, grade 2 at 1 hour and grade 1 at 24 hours), discharge (1/3 females, grade 2 at 1 hour), and iritis (1/3 female rabbits, grade 1) were noted for the washed eye group. Blistering of the conjunctivae was also observed at the 1 hour evaluation in 3/3 female rabbits. Ocular irritation had subsided in the washed eye group of females rabbits by the 48 hour evaluation interval. Incidences of redness, chemosis, discharge and iritis resulted in Primary Irritation Scores (P.I.S.) of 9.0 and 4.0 for unwashed eyes at 1 and 24 hours, respectively and 3.2 for washed eyes at 1 hour.

In this primary eye irritation study, Enfrost (urea, 42.9% a.i.) produces minimal irritation (clearing within 24 hours in unwashed eyes and within 48 hours in washed eyes) and is assigned to TOXICITY CATEGORY IV.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a primary eye irritation study [870.2400 (§81-4)] in rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and [No] Data Confidentiality statements were provided.

5. STUDY TYPE: Primary Dermal Irritation - Rabbit [OPPTS 870.2500 (§81-5)]

CITATION: Smith, Sandra (1986): Primary Dermal Irritation in Rabbits with Unocal Plus. American Biogenics Corporation, 1800 East Pershing Road, Decatur, IL 62526. Study Number ID: 480-2779, September 12, 1986. MRID 40733306. Unpublished.

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 40733306), New Zealand white rabbits (6 males) were dermally exposed to 0.5 ml of Enfrost (urea, 42.9% a.i.) for 4 hours on approximately 6 cm^2 of the dorsum of each animal. Rabbits were observed for dermal irritation at 0.5, 1.0, 24, 48, and 72 hours following test material removal. Each test site was scored for dermal irritation using a grading system for dermal lesions that is comparable to the Draize Dermal Scoring System. Body weights were measured prior to study initiation and at study termination (test day 7). The animals were observed for clinical signs of toxicity for 7 days post-dosing.

Within 30 to 60 minutes of test compound removal, very slight erythema (grade 1) occurred in 4/6 male rabbits at one or both abraded skin sites and at both intact skin sites in one of the four affected rabbits. At 24 hours, two of these rabbits continued to exhibit very slight erythema (grade 1, two abraded skin sites), whereas, at 48 hours, very slight erythema (grade 1, one abraded skin site) persisted in only one of these animals. Edema was not observed in any rabbit tested in this study and signs of dermal irritation (erythema) observed at earlier evaluations were no longer present at 72 hours. All animals survived until study termination and necropsy revealed no gross pathologies.

In this primary dermal irritation study, Enfrost is a slight skin irritant. The calculated Primary Irritation Score is 0.2 (minimally irritating). Enfrost (urea, 42.9% a.i.) is assigned to TOXICITY CATEGORY IV based upon dermal irritation in male rabbits.

This acute toxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a primary dermal irritation study [870.2500 (§81-5)] in rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and [No] Data Confidentiality statements were provided.

6. STUDY TYPE: Skin Sensitization - Guinea Pig [OPPTS 870.2600 (§81-6)]

CITATION: Smith, Sandra (1986): Dermal Sensitization Study in Guinea Pigs with Unocal Plus. American Biogenics Corporation, 1800 East Pershing Road, Decatur, IL 62526. Study Number ID: 480-2781, September 24, 1986. MRID 40733303. Unpublished.

87

EXECUTIVE SUMMARY: In a skin sensitization study (MRID 40733303), groups of Hartley Albino guinea pigs (22 females) were tested using the Buehler Topical Closed-patch Technique. In a range-finding study, 2 female guinea pigs received dermal applications of Enfrost (urea, 42.9% a.i.) at concentrations of 3, 10, 30% in deionized water and 100% (undiluted) for a 6 hour exposure duration. For the induction phase of the study, 10 females guinea pigs received 100% Enfrost as multiple dermal applications (three times weekly for three consecutive weeks; 9 induction applications). For the challenge phase of the study, 100% test material was applied dermally to these animals two weeks after the final induction exposure. A challenge control group (animals not treated during the induction phase) consisting of 10 female guinea pigs was tested concurrently. Each test site was examined for at 24 and 48 hours post-application (induction and challenge) and graded for dermal irritation (erythema, edema and other lesions using the Draize Dermal Grading System. Individual body weights were determined for test and challenge control guinea pigs on the day of the initial induction application (day 0) and on the day of the 48-hour challenge evaluations (day 35). All animals were observed for mortality and abnormal clinical signs at least once daily during the testing period and any animal found dead on study was subjected to gross pathological examination.

All surviving animals were free of abnormal clinical signs and gained an appropriate amount of weight for the duration of the study. One guinea pig in the test group was found dead on day 22 of the study. Necropsy examination of this animal revealed the following: abdominal cavity filled with red fluid and clotted material, discolored dark red ovaries and a pale appearance to all other tissues. The study authors did not provide an explanation as to the cause of this death. Although the preliminary range-finding trials (2 guinea pigs) revealed no erythema or edema at any of the concentrations of the test material evaluated (3, 10, 30, and 100%), undiluted Unocal Plus (100% concentration) was utilized in the main study to test the potential for dermal hypersensitivity reactions. In the main study, very slight erythema was noted at the test sites of two animals 24 hours after the first induction application. Very slight erythema (grade 1) and/or edema (grade 1) was observed in one of these two animals at both the 24 and 48 hour evaluation intervals after induction applications 6, 7, 8, and 9. Following the challenge application of test material, no dermal reactions were noted among either test guinea pigs or the challenge control group animals. The study report included positive control data using a known dermal sensitizer, dinitrochlorobenzene (DCNB) prepared in 0.1% acetone. These data were obtained within one year of the current study and the results were appropriate.

In this skin sensitization study, Enfrost (urea, 42.9% a.i.) was not a skin (dermal) sensitizer.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a dermal sensitization study [870.2600 (§81-6)] in guinea pigs.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and [No] Data Confidentiality statements were provided.

88

cc (with attachments): Michelle Centra (RRB III). Catherine Eiden (RRB III). Joseph Nevola (SRRD. RRB II). Robert McNally (SRRD. RRB II).

EPA Primary Reviewer: Michelle M. Centra
Reregistration Branch III (7509C)
EPA Secondary Reviewer: Stephen C. Dapson
Registration Action Branch III (7509C)

Michelle M. Centra Date 5/23/01

Stephen C. Dapson Date 06/09/2001

TXR Number: 0050171

REVISED DATA EVALUATION RECORD

This addendum provides a revised Executive Summary to the original Data Evaluation Record in TXR No. 007297 and 008416 for MRID No. 40733304

STUDY TYPE: Acute Oral Toxicity - Rat [OPPTS 870.1100 (§81-1)]

EPA ID NUMBERS: PC Code: 085702
DP Barcode: D277687
Submission No.: S596788
MRID No.: 40733304
PRAT Case No.: 819300
Reregistration Case No.: 4096
CAS No.: 57-13-6

TEST MATERIAL (PURITY): Enfrost (urea, 42.9% a.i.)

SYNONYMS: Urea, Unocal Plus

CITATION: Smith, Sandra (1986): Acute Oral Toxicity Study with Rats with Unocal Plus. American Biogenics Corporation, 1800 East Pershing Road, Decatur, IL 62526. Study Number ID: 480-2777, September 24, 1986. MRID 40733304. Unpublished.

SPONSOR: Unocal Corporation, Los Angeles, CA 90051

EXECUTIVE SUMMARY: In an acute oral (limit dose) toxicity study (MRID 40733304) five male and five female fasted, young adult, Sprague-Dawley rats received a single oral gavage dose of 5000 mg/kg Enfrost (urea, 42.9% a.i.). Body weights were measured prior to test material administration (day 0), on day 7, and prior to sacrifice on day 14. Animals were observed at least once daily for mortality and abnormal clinical signs throughout the study and subjected to gross pathology examination on day 14.

All animals survived until study termination and gained an appropriate amount of weight throughout the observation period. Clinical signs of toxicity were not observed during the study and necropsy evaluation of all animals revealed no abnormal findings.

The acute oral LD_{50} value for male and female rats is greater than 5000 mg/kg (limit dose). Enfrost (urea, 42.9% a.i.) is assigned to TOXICITY CATEGORY IV based on the oral LD_{50} value established in this study.

This acute oral study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for an acute oral (limit dose) toxicity study [870.1100 (§81-1)] in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and [No] Data Confidentiality statements were provided.

Reviewed by: Sidney Stolzenberg, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Michael Ioannou, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C)

S J Stolzenberg 6/12/89
M J 6-13-89

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral toxicity

GUIDELINE: 81-1

TEST MATERIAL: Unocal Plus

SYNONYMS: Urea, Enfrost

STUDY NUMBER(S): 480-2777

Caswell No. 902

HED Project No. 8-1038

MRID No. 407333-04

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TESTING FACILITY: American Biogenics Corp.
Decatur, IL 62526

TITLE OF REPORT: Acute Oral Toxicity Study in Rats with Unocal Plus

AUTHOR(S): Sandra Smith

REPORT ISSUED: Sept. 24, 1986

CONCLUSIONS AND RECOMMENDATIONS

Classification: Core Supplementary

The purity of urea used and the percentage of urea in the test substance were not indicated. This study may be upgraded when this information is received. The following values should be assigned if upgraded.

LD50 > 5 g/kg (Limit dose test). Tox. Category IV

A. MATERIALS

1. Test Compound: Unocal Plus. Description: A liquid. Purity, contaminants and inert material composition are not listed. Density of substance was determined to be 1113.1 mg/ml

2. Test animals: "Young adult" Sprague-Dawley rats from Charles River in Portage, MI. Body weight on day 0 of the study was 262 ± 18 g for males, 201 ± 5 g for the females.

B. PROCEDURES

Five of each sex received 5 g/kg, (limit dose test) by oral gavage. Body weights were obtained on days 0, 7 and 14. Necropsies were performed under supervision of a pathologist. Animals were observed for clinical signs throughout the study.

C. COMPLIANCE

- A signed Quality Assurance statement was included.

D. RESULTS

No effect of treatment was observed.

E. CONCLUSION:

Classification: Core Supplementary

The purity of urea and composition of urea in the test substance were not indicated. This classification may be upgraded when this information is supplied. The following values are recommended if the study is upgraded.

LD_{50} > 5 g/kg Tox. Category IV

EPA Primary Reviewer: Michelle M. Centra
Reregistration Branch III (7509C)
EPA Secondary Reviewer: Stephen C. Dapson
Registration Action Branch III (7509C)

Michelle M. Centra, Date 5/23/01

Stephen C. Dapson, Date 06/09/2001

TXR Number: 0050171

REVISED DATA EVALUATION RECORD

This addendum provides a revised Executive Summary to the original Data Evaluation Record in TXR No. 007297 and 008416 for MRID No. 40733305

STUDY TYPE: Acute Dermal Toxicity - Rabbit [OPPTS 870.1200 (§81-2)]

EPA ID NUMBERS: PC Code: 085702
DP Barcode: D277687
Submission No.: S596788
MRID No.: 40733305
PRAT Case No.: 819300
Reregistration Case No.: 4096
CAS No.: 57-13-6

TEST MATERIAL (PURITY): Enfrost (urea, 42.9% a.i.)

SYNONYMS: Urea, Unocal Plus

CITATION: Smith, Sandra (1986): Acute Dermal Toxicity Study in Rabbits with Unocal Plus. American Biogenics Corporation, 1800 East Pershing Road, Decatur, IL 62526. Study Number ID: 480-2778, September 23, 1986. MRID 40733305. Unpublished.

SPONSOR: Unocal Corporation, Los Angeles, CA 90051

EXECUTIVE SUMMARY: In an acute (limit dose) dermal toxicity study (MRID 40733305), New Zealand white rabbits (5/sex) were dermally exposed to a single dose of 2000 mg/kg Enfrost (urea, 42.9% a.i.) for 24 hours. Body weights were measured prior to test article application (study day 0), on study day 7, and prior to sacrifice (study day 14). The animals were observed for clinical signs of toxicity throughout the study and subjected to gross pathology examination on day 14.

At 2000 mg/kg, treatment-related erythema (skin reddening) occurred in 8/10 rabbits (3/5 males and 5/5 females) on day 2 of the study. Dermal irritation was resolved in all of the affected animals by study day 3. All animals survived the 14 day observation period and gained an appropriate amount of body weight throughout the study. There were no clinical signs of toxicity noted and necropsy revealed no gross pathologies in any animal treated with 2000 mg/kg test compound.

The dermal LD_{50} value for male and female rabbits was greater than 2000 mg/kg. Enfrost (urea, 42.9% a.i.) is assigned to TOXICITY CATEGORY III based upon the dermal (limit dose) LD_{50} value established in this study.

04

This acute dermal study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for an acute dermal toxicity study [870.1200 (§81-2)] in rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Statement of Compliance and [No] Data Confidentiality statements were provided.

Reviewed by: Sidney Stolzenberg, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Michael Ioannou, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C)

S Stolzenberg 6/12/89
M F 6-13-89

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity

Caswell No. 902

GUIDELINE: 81-2

HED Project No. 8-1038

TEST MATERIAL: Unocal Plus

MRID No. 407333-05

SYNONYMS: Urea, Enfrost

STUDY NUMBER(S): 480-2778

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TESTING FACILITY: American Biogenics Corp.
Decatur, IL. 62526

TITLE OF REPORT: Acute Dermal Toxicity Study in Rabbits with Unocal Plus.

AUTHOR(S): Sandra Smith

REPORT ISSUED: Sept. 23, 1986

CONCLUSIONS AND RECOMMENDATIONS

Core Classification: Supplementary

The purity of urea and composition of urea in the test substance were not indicated. The classification may be upgraded when this information is supplied. The following values are recommended if this study is upgraded.

LD50 > 2 g/kg --Tox. Category III

96

A. MATERIALS

1. Test compound: Unocal Plus. Description: Liquid, Lot No. AO-4201, density determined to be 1113.1 mg/ml

2. Test animals: New Zealand White young rabbits (age not specified), males and females, weighing 2.5 to 3.2 kg at the start of the study.

3. Dosage: Neat substance, 2 g/kg body weight, applied to shared skin on about 10% of body surface with about 22.8 mg/cm^2 of body surface.

B. STUDY DESIGN AND METHODS

Five of each sex were used. Test substance was held in place with plastic wrapper impervious to compound. The compound was removed after 24 hours of skin contract. Observation period was for 14 days with body weights obtained on days 0, 7 and 14. Animals were subjected to gross pathology on day 14.

C. COMPLIANCE

- A signed statement of Quality Assurance was provided.

D. RESULTS

No effect on clinical signs, observed each day, no effect on weight gain; all animals survived to day 14. Erythema was present on day 2 in 3 males and all 5 females. No abnormalities were observed at necropsy.

E. CONCLUSION

Core Classification: Supplementary

The purity of urea and composition of urea in the test substance were not indicated. The classification may be upgraded when this information is supplied. The following values are recommended if this study is upgraded.

LD50 > 2 g/kg Tox. Category III

EPA Primary Reviewer: Michelle M. Centra
Reregistration Branch III (7509C)
EPA Secondary Reviewer: Stephen C. Dapson
Registration Action Branch III (7509C)

Michelle M. Centra. Date 5/23/01

Stephen C Dapson. Date 06/09/2001

TXR Number: 0050171

REVISED DATA EVALUATION RECORD

This addendum provides a revised Executive Summary to the original Data Evaluation Record in TXR No. 007297 and 008416 for MRID No. 40733301

STUDY TYPE: Acute Inhalation Toxicity - Rat [OPPTS 870.1300 (§81-3)]

EPA ID NUMBERS: PC Code: 085702
DP Barcode: D277687
Submission No.: S596788
MRID No.: 40733301
PRAT Case No.: 819300
Reregistration Case No.: 4096
CAS No.: 57-13-6

TEST MATERIAL (PURITY): Enfrost (urea, 42.5% a.i.)

SYNONYMS: Urea, Unocal Plus

CITATION: Smith, Sandra (1986): Acute Inhalation Toxicity Study with Rats with Unocal Plus. American Biogenics Corporation, 1800 East Pershing Road, Decatur, IL 62526. Laboratory ID Number: Study I-7090.112, May 11, 1988. MRID 40733301. Unpublished.

SPONSOR: Unocal Corporation, Los Angeles, CA 90051

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 40733301), Fischer 344 rats (5/sex) were exposed "nose only" to the highest attainable nominal concentration (4.8 mg/L) of Enfrost (urea, 42.5% a.i.) for 4 hours. A sham control group (used to control for the biological effects of restraint) consisting of 5 male and 5 female rats was exposed to air alone for 4 hours. Flow rate was set at 0.5 L/min and the Mass Median Aerodynamic Diameter (MMAD) for the 4 hour Enfrost exposure ranged from 2.4 to 3.1 microns (average: 2.75 microns). Body weights were measured prior to treatment and once weekly thereafter. The animals were observed for mortality and clinical signs at 1 and 3 hours following exposure, then once daily thereafter for the duration of the study period (14 days). Complete necropsies were performed on all animals on day 15 of the study.

Eye tearing was observed in both treated (5/5 males, 5/5 females) and sham control (5/5 males, 5/5 females) animals during exposure and at the one and three hour post-exposure evaluation intervals. All animals (including the sham controls) presented with hunched posture, ruffled and soiled fur (stained brown) and red colored secretions from the eyes (tearing) at one hour post-treatment. A red colored secretion from the nose was also observed in treated rats (5/5 males, 5/5

females) one hour after exposure, however, nasal secretions were no longer evident by 3 hours. Rats appeared normal by 24 hours post-treatment and no unusual behavior or appearance was observed for the remainder of the test period (14 days). There were no mortalities and no significant differences in body weight/body weight gain between treated and sham control groups. No gross lesions were observed at necropsy.

Based on these results, the LC_{50} for Unocal Plus (Urea, 45.3% a.i.) is greater than 4.8 mg/L.

This acute inhalation study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for an acute inhalation toxicity study [870.1300 (§81-3)] in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Statement of Compliance and [No] Data Confidentiality statements were provided.

007297

Reviewed by: Sidney Stolzenberg, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Michael Ioannou, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C)

S Stolzenberg 6/12/89
M.I 6/12/89

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation

Caswell No. 902

GUIDELINE: 81-3

HED Project No. 8-1038

TEST MATERIAL: Unocal Plus

MRID No. 407333-01

SYNONYMS: Urea, Enfrost

Lab. STUDY NUMBER(S): I-7090.112

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TESTING FACILITY: Microbiological Associates
Bethesda, MD 20816

TITLE OF REPORT: Acute Inhalation Toxicity Study of Unocal Plus in the Rat.

AUTHOR(S): R.M. David, PhD., DABT

REPORT ISSUED: May 11, 1988

CONCLUSIONS AND RECOMMENDATIONS

The substance tested is considered to be Enfrost, previously called Unocal Plus. This product has a urea content of 42.5%.

Inhalation LC_{50} > 4.8 mg/L/4 hours. Tox. Category III

Core Classification: Minimum

100

A. MATERIALS

1. Test compound: Unocal Plus. Description: Yellow liquid. Lot: Reference No. AP 4186, test article no. T07090A. Purity: 42.5% urea. Contaminants and inert materials were not listed.

2. Test animals: Rats, Fischer 344, 9 week old when treated; body weights were 193-217 g for males, 138-160 g for females, on treatment day.

B. STUDY DESIGN AND METHOD

Five males and 5 females were exposed to a dose of 4.8 mg/L nominal concentration, the highest attainable concentration of the aerosolized test substance. A Collision Nebulizer was used to generate the aerosol. There was no mention of temperature or solvent used to assist in vaporization of the test substance. Nose only exposure units were used. Five animals were restrained per unit exposure. Air flow was approximately 5L/min, which was periodically monitored. Sampling of test atmosphere was done from a sampling port situated on the side of the exposure module (apparently at only one location). Samples were analyzed for gravimetric and particle size determinations. Five separate determinations were made and a time-weighted average was calculated for total aerosol concentration. Particle size was determined by using a Mercer 7 stage cascade impactor with cutoff stages for 7 different particle sizes ranging from 0.5 to 10 micron. Oxygen determinations were made in the chamber and set at 20% throughout the exposure period. Samples from the neat material and from the atmosphere were analyzed for urea. A "sham exposed" control group received the same restraint in the exposure chamber as the treated rats with the same air flow rate. Exposure period was for 4 hours.

Observations included clinical signs at 1 and 3 hours post-exposure, then once daily thereafter to day 14. Complete necropsies were performed on day 15 when organs with lesions were preserved. Body weights were obtained at pre-dose, then weekly.

C. COMPLIANCE

- A signed statement of compliance with EPA's GLP was provided.

D. Results

The highest concentration of Unocal Plus actually attained in the chamber, indicated in the text, was 3.5 mg/L. Analytical concentration of urea in test atmosphere was 70 %, compared to 48.5% urea in the neat material. No table of results for these determinations were found. Average for mass median diameter was 2.75 microns, based on 2 determinations in which the actual results came to 2,4 and 3.1 microns.

Respirable concentration (< 15 microns), based on size distribution, was 95-99% of the 4.8 mg/L.

Animal Observations.

Mortality: None

Cinical signs: Red color secretions from the nose, observed on at 1 hour after exposure, was not seen at 3 hours or after. This was the only compound related effect.

Body weights: No compound related effect.

Gross pathology: No gross lesions were observed.

The LC_{50} is considered to be > 4.8 mg/L/4 hours

EPA Primary Reviewer: Michelle M. Centra
Reregistration Branch III (7509C)
EPA Secondary Reviewer: Stephen C. Dapson
Registration Action Branch III (7509C)

Michelle M. Centra Date 5/23/01
Stephen C. Dapson Date 06/09/2001

TXR Number: 0050171

REVISED DATA EVALUATION RECORD

This addendum provides a revised Executive Summary to the original Data Evaluation Record in TXR No. 007297 and 008416 for MRID No. 40733302

STUDY TYPE: Primary Eye Irritation - Rabbit [OPPTS 870.2400 (§81-4)]

EPA ID NUMBERS: PC Code: 085702
DP Barcode: D277687
Submission No.: S596788
MRID No.: 40733302
PRAT Case No.: 819300
Reregistration Case No.: 4096
CAS No.: 57-13-6

TEST MATERIAL (PURITY): Enfrost (urea, 42.9% a.i.)

SYNONYMS: Urea, Unocal Plus

CITATION: Smith, Sandra (1986): Primary Eye Irritation Study in Rabbits with Unocal Plus. American Biogenics Corporation, 1800 East Pershing Road, Decatur, IL 62526. Study Number ID: 480-2780, September 12, 1986. MRID 40733302. Unpublished.

SPONSOR: Unocal Corporation, Los Angeles, CA 90051

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 40733302), New Zealand white rabbits (six males and three females) received 0.1 ml of Enfrost (urea, 42.9% a.i., undiluted) in the lower lid of the right eye. The treated eyes of three female rabbits were rinsed with warm tap water for 30 seconds following instillation of the test substance while the treated eyes of six male rabbits were left unwashed (the left eye remained untreated and served as the control). Each treated eye was evaluated for ocular irritation and lesions using fluorescein stain at 24, 48, and 72 hours (the 1 hour evaluation time period was performed without fluorescein stain). Eye irritation ratings were determined according to the method of Kay and Calandra (J. Soc. Cosmetic Chemists 13: 281-289, 1962), which uses a Draize scoring system. Animals were observed for mortality and clinical signs at least once daily during the study period (72 hours).

No mortalities occurred and no abnormal clinical signs were observed during the study. At the1 hour evaluation interval, animals in the unwashed eye group exhibited redness (5/6 males, grade 1), chemosis (2/6 males, grade1), and iritis (1/6 males, grade 1). All ocular irritation occurring in the unwashed eyes of male rabbits was resolved by 24 hours. Redness (3/3 females, grade 1 at both the 1 and 24 hour evaluation intervals), chemosis (3/3 females, grade 2 at 1 hour and grade

1 at 24 hours), discharge (1/3 females, grade 2 at 1 hour), and iritis (1/3 female rabbits, grade 1) were noted for the washed eye group. Blistering of the conjunctivae was also observed at the 1 hour evaluation in 3/3 female rabbits. Ocular irritation had subsided in the washed eye group of females rabbits by the 48 hour evaluation interval. Incidences of redness, chemosis, discharge and iritis resulted in Primary Irritation Scores (P.I.S.) of 9.0 and 4.0 for unwashed eyes at 1 and 24 hours, respectively and 3.2 for washed eyes at 1 hour.

In this primary eye irritation study, Enfrost (urea, 42.9% a.i.) produces minimal irritation (clearing within 24 hours in unwashed eyes and within 48 hours in washed eyes) and is assigned to TOXICITY CATEGORY IV.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a primary eye irritation study [870.2400 (§81-4)] in rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and [No] Data Confidentiality statements were provided.

104

007297

Reviewed by: Sidney Stolzenberg, Ph.D. S J Stolzenberg 6/12/89
Section I, TOX Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Michael Ioannou, Ph.D. M.I. 6-13-89
Section I, TOX Branch II, HFAS/HED (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irritation

Caswell No. 902

GUIDELINE: 81-4

HED Project No. 8-1038

TEST MATERIAL: Unocal Plus

MRID No. 407333-02

SYNONYMS: Urea, Enfrost

STUDY NUMBER(S): 480-2780

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TESTING FACILITY: American Biogenics Corp
Decatur, IL. 62526

TITLE OF REPORT: Primary Eye Irritation Study in Rabbits with Unocal Plus.

AUTHOR(S): S. H. Smith

REPORT ISSUED: June 25, 1988

CONCLUSIONS (Summary)

Classification: Core Supplementary

The purity of urea and content of urea in the test substance were not stated in the report. The following P.I.S. values and Toxicity Category are recommended if the study is upgraded.

	Unwashed	Washed
P.I.S. at 1 Hour:	3.2	9.0
24 Hours:	0.0	4.0
48 Hours:	0.0	0.0

Tox Category IV for primary eye irritation; minimallly irritating, clearing within 24 hours in unwashed eye, within 48 hours in washed eye.

105

A. MATERIALS

1. Test compound: Unocal Plus. Description: Liquid, pH 6.0 approximately. Lot No. A04201. Contaminants are not listed; stored at ambient laboratory conditions.

2. Test animals: Rabbits, New Zealand White, obtained from Clerco Research Farm in Cincinnati, OH. "Young Adult" animals were used but their age or body weight were not indicated.

B. STUDY

Dosage: 0.1 ml, without dilution.

Route: Instilled into the everted lower lid of the right eye of each animal. The eyelids were held together for about 1 second.

C. METHODS: Six males on test had no washing of eye after dosing. Three females had the treated eye washed with warm tap water for one minute at a rate of approximately one liter/minute about 30 seconds after instillation of test substance into the eye. The treated eye was examined for irritation and lesions at 1, 24, 48 and 72 hours. Fluorescein, 2% solution, was used as an aid for evaluation at all time periods, except for the one hour period. Eye irritation ratings were determined according to the method of Kay and Calandra (J. Soc. Cosmetic Chemists 13: 281-289, 1962), which uses a Draize scoring system.

D. COMPLIANCE:

- A signed Quality Assurance Statement was provided.

E. RESULTS

The following are mean values for the six unwashed and three washed treated eyes, calculated from the individual animal results of Tables 2 and 3 in the submitted report.

	1 Hour		24 Hour		48 Hour		72 Hour	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
I. Cornea								
A. Density	0	0	0	0	0	0	0	0
B. Area	0	0	0	0	0	0	0	0
II. Iris	0.2	0.3	0	0.3	0	0	0	0
III. Conjunctivae								
A. Redness	0.8	1.0	0	1.0	0	0	0	0
B. Chemosis	0.3	2.0	0	1.0	0	0	0	0
C. Discharge	0	0.7	0	0	0	0	0	0

F. CONCLUSION:

Classification: Core Supplementary

There is no information on purity of urea or ccontent of urea in the test substance. The following P.I.S. values and Toxicity categories are recommended if this study is upgraded.

	Unwashed	Washed
P.I.S. at 1 hours:	3.2	9.0
24 hours:	0.0	4.0
48 hours:	0.0	0.0
72 hours:	0.0	0.0

Tox Category IV for primary eye irritation (minimally irritating, clearing within 24 hours for unwashed eye 48 hours for washed eye).

157

EPA Primary Reviewer: Michelle M. Centra
Reregistration Branch III (7509C)
EPA Secondary Reviewer: Stephen C. Dapson
Registration Action Branch III (7509C)

Michelle M. Centra, Date 5/23/01
Stephen C. Dapson, Date 06/09/2001

TXR Number: 0050171

REVISED DATA EVALUATION RECORD

This addendum provides a revised Executive Summary to the original Data Evaluation Record in TXR No. 007297 and 008416 for MRID No. 40733306

STUDY TYPE: Primary Dermal Irritation - Rat [OPPTS 870.2500 (§81-5)]

EPA ID NUMBERS: PC Code: 085702
DP Barcode: D277687
Submission No.: S596788
MRID No.: 40733306
PRAT Case No.: 819300
Reregistration Case No.: 4096
CAS No.: 57-13-6

TEST MATERIAL (PURITY): Enfrost (urea, 42.9% a.i.)

SYNONYMS: Urea, Unocal Plus

CITATION: Smith, Sandra (1986): Primary Dermal Irritation in Rabbits with Unocal Plus. American Biogenics Corporation, 1800 East Pershing Road. Decatur, IL 62526. Study Number ID: 480-2779, September 12, 1986. MRID 40733306. Unpublished.

SPONSOR: Unocal Corporation, Los Angeles, CA 90051

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 40733306), New Zealand white rabbits (6 males) were dermally exposed to 0.5 ml of Enfrost (urea, 42.9% a.i.) for 4 hours on approximately 6 cm^2 of the dorsum of each animal. Rabbits were observed for dermal irritation at 0.5, 1.0, 24, 48, and 72 hours following test material removal. Each test site was scored for dermal irritation using a grading system for dermal lesions that is comparable to the Draize Dermal Scoring System. Body weights were measured prior to study initiation and at study termination (test day 7). The animals were observed for clinical signs of toxicity for 7 days post-dosing.

Within 30 to 60 minutes of test compound removal, very slight erythema (grade 1) occurred in 4/6 male rabbits at one or both abraded skin sites and at both intact skin sites in one of the four affected rabbits. At 24 hours, two of these rabbits continued to exhibit very slight erythema (grade 1, two abraded skin sites), whereas, at 48 hours, very slight erythema (grade 1, one abraded skin site) persisted in only one of these animals. Edema was not observed in any rabbit tested in this study and signs of dermal irritation (erythema) observed at earlier evaluations were no longer present at 72 hours. All animals survived until study termination and necropsy

revealed no gross pathologies.

In this primary dermal irritation study, Enfrost is a slight skin irritant. The calculated Primary Irritation Score is 0.2 (minimally irritating). Enfrost (urea, 42.9% a.i.) is assigned to TOXICITY CATEGORY IV based upon dermal irritation in male rabbits.

This acute toxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a primary dermal irritation study [870.2500 (§81-5)] in rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Statement of Compliance and [No] Data Confidentiality statements were provided.

007297

Reviewed by: Sidney Stolzenberg, Ph.D. S.J. Stolzenberg 6/12/89
Section I, TOX Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Michael Ioannou, Ph.D. J.M.I 6-13-89
Section I, TOX Branch II, HFAS/HED (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Primary Dermal Irritation

Caswell No. 902

GUIDELINE: 81-5

HED Project No. 8-1038

TEST MATERIAL: Unocal Plus

MRID No. 407333-06

SYNONYMS: Urea, Enfrost

STUDY NUMBER(S): 480-2779

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TESTING FACILITY: American Biogenics Corp
Decatur, IL 62526

TITLE OF REPORT: Primary Dermal Irritation in Rabbits with Unocal Plus.

AUTHOR(S): Sandra Smith

REPORT ISSUED: Sept. 12, 1986

CONCLUSIONS AND RECOMMENDATIONS

Classification: Core Supplementary

There is no information on purity of urea and composition of urea in the test substance. The study should be upgraded if this information is supplied and the following values assigned.

Mean P.I.S Scores: 0.2 at 30-60 minutes
0 at 72 hours

Tox. Category IV. Minimally irritating

110

A. MATERIALS

1. Test compound: Unocal Plus. A liquid, Lot # A04201; pH 6.0. Purity was not indicated.

2. Test animals: New Zealand Albino rabbits "young adult", weights are not indicated. Obtained from Clerco Research Farm, Cincinnati, OH.

B. STUDY PROCEDURE

Six male rabbits were shaved, at 4 different sites per rabbit, on each side of the thoracic region of the spinal column. Two shaved sites, both on the right side were abraded, the other 2 sites were left intact. 0.5 ml of neat test substance was applied by means of a 2.5 cm^2 gauze patch, to each of the 4 prepared test sites on all 6 rabbits. After 4 hours of exposure, test substance was removed from each test site and evaluated for erythema, edema and other possible lesions at 30-60 minutes, 24, 48 and 72 hours.

C. COMPLIANCE

- A signed statement of Quality Assurance was submitted.

D. Results

No mortalities or clinical signs were observed.

PIS Scores:

Time	Mean Score	
30-60 min	0.1	Minimally irritating
24 hours	0.1	
48 hours	0	
72 hours	0	

E. CONCLUSION

Classification: Core Supplementary

The purity of urea and composition of urea in the test substance were not stated in the report. The study may be upgraded when the information is supplied.

Tox. Category: IV

This value should be assigned if the study is upgraded.

111

EPA Primary Reviewer: Michelle M. Centra
Reregistration Branch III (7509C)
EPA Secondary Reviewer: Stephen C. Dapson
Registration Action Branch III (7509C)

Michelle M. Centra Date 5/23/01
Stephen C. Dapson Date 06/09/2001

TXR Number: 0050171

REVISED DATA EVALUATION RECORD

This addendum provides a revised Executive Summary to the original Data Evaluation Record in TXR No. 007297 and 008416 for MRID No. 40733303

STUDY TYPE: Skin Sensitization - Guinea Pig [OPPTS 870.2600 (§81-6)]

EPA ID NUMBERS: PC Code: 085702
DP Barcode: D277687
Submission No.: S596788
MRID No.: 40733303
PRAT Case No.: 819300
Reregistration Case No.: 4096
CAS No.: 57-13-6

TEST MATERIAL (PURITY): Enfrost (urea, 42.9% a.i.)

SYNONYMS: Urea, Unocal Plus

CITATION: Smith, Sandra (1986): Dermal Sensitization Study in Guinea Pigs with Unocal Plus. American Biogenics Corporation, 1800 East Pershing Road, Decatur, IL 62526. Study Number ID: 480-2781, September 12, 1986. MRID 40733303. Unpublished.

SPONSOR: Unocal Corporation, Los Angeles, CA 90051

EXECUTIVE SUMMARY: In a skin sensitization study (MRID 40733303), groups of Hartley Albino guinea pigs (22 females) were tested using the Buehler Topical Closed-patch Technique. In a range-finding study, 2 female guinea pigs received dermal applications of Enfrost (urea, 42.9% a.i.) at concentrations of 3, 10, 30% in deionized water and 100% (undiluted) for a 6 hour exposure duration. For the induction phase of the study, 10 females guinea pigs received 100% Enfrost as multiple dermal applications (three times weekly for three consecutive weeks; 9 induction applications). For the challenge phase of the study, 100% test material was applied dermally to these animals two weeks after the final induction exposure. A challenge control group (animals not treated during the induction phase) consisting of 10 female guinea pigs was tested concurrently. Each test site was examined for at 24 and 48 hours post-application (induction and challenge) and graded for dermal irritation (erythema, edema and other lesions using the Draize Dermal Grading System. Individual body weights were determined for test and challenge control guinea pigs on the day of the initial induction application (day 0) and on the day of the 48-hour challenge evaluations (day 35). All animals were observed for mortality and abnormal clinical signs at least once daily during the testing period and any animal found dead on study was subjected to gross pathological examination.

All surviving animals were free of abnormal clinical signs and gained an appropriate amount of weight for the duration of the study. One guinea pig in the test group was found dead on day 22 of the study. Necropsy examination of this animal revealed the following: abdominal cavity filled with red fluid and clotted material; discolored dark red ovaries and a pale appearance to all other tissues. The study authors did not provide an explanation as to the cause of this death. Although the preliminary range-finding trials (2 guinea pigs) revealed no erythema or edema at any of the concentrations of the test material evaluated (3, 10, 30, and 100%), undiluted Unocal Plus (100% concentration) was utilized in the main study to test the potential for dermal hypersensitivity reactions. In the main study, very slight erythema was noted at the test sites of two animals 24 hours after the first induction application. Very slight erythema (grade 1) and/or edema (grade 1) was observed in one of these two animals at both the 24 and 48 hour evaluation intervals after induction applications 6, 7, 8, and 9. Following the challenge application of test material, no dermal reactions were noted among either test guinea pigs or the challenge control group animals. The study report included positive control data using a known dermal sensitizer, dinitrochlorobenzene (DCNB) prepared in 0.1% acetone. These data were obtained within one year of the current study and the results were appropriate.

In this skin sensitization study, Enfrost (urea, 42.9% a.i.) was not a skin (dermal) sensitizer.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a dermal sensitization study [870.2600 (§81-6)] in guinea pigs.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and [No] Data Confidentiality statements were provided.

113

Reviewed by: Sidney Stolzenberg, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Michael Ioannou, Ph.D.
Section I, TOX Branch II, HFAS/HED (H7509C)

S Stolzenberg 6/1/89
M.I. 6-13-89

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization

GUIDELINE: 81-6

TEST MATERIAL: Unocal Plus

SYNONYMS: Urea, Enfrost

STUDY NUMBER(S): 480-2781

Caswell No. 902

HED Project No. 8-1038

MRID No. 407333-03

SPONSOR: Unocal Corporation
Los Angeles, CA 90051

TESTING FACILITY: American Biogenics Corp
Decatur, IL 62526

TITLE OF REPORT: Dermal Sensitization Study in Guinea Pigs with Unocal Plus

AUTHOR(S): Sandra Smith

REPORT ISSUED: Sept. 24, 1986

CONCLUSIONS AND RECOMMENDATIONS

No skin sensitization was seen.

Core Classification: Supplementary

The purity of urea and percentage of urea in the test substance was not indicated. The classification may be upgraded when this information is supplied.

A. MATERIALS

1. Test Compound: Unocal Plus. Description: A liquid. Lot # AO-420. Purity of urea and percentage of urea in the test substance were not listed.

2. Test animals: Guinea pigs, Hartley strain, young adults; age and weight were not specified. Obtained from Harlan Sprague Dawley, Inc., Indianapolis, IN.

B. PROCEDURES

Range Finding Study: Two guinea pigs received 0.5 ml of varied concentrations, diluted by suspension in deionized water. There was a 6 hour exposure duration for each application. Two concentrations were evaluated per animal, using suspensions of 3, 10, 30 and 100%.

Main Study: Consisted of 2 groups of 10 animals. In the test group, 9 induction applications of 0.5 ml undiluted test substance was applied to the shaved skin on the back of flank, 3 times per week (3 weeks); 6 hour exposure period for each application of 0.5 ml undiluted test material was placed on the shaved skin. The second group of test animals was a "naive" control group. Animals in naive control group received no induction applications but received only the challenge dose of 0.5 ml for a 6 hour exposure period. A 4X4 cm Webril patch was used to apply the test substance for each induction and challenge application.

After removal of the patch, the sites on each animal were examined for erythema, edema and other lesions 24 hours after each application. Evaluation by a grade of 0 to 4 according to Draize scoring systems for erythema and for edema were used 24 and 48 hours after each application.

There was no concomitant positive control with this study. Instead, an historic positive control, performed in November, 1985, was used for comparison. For this positive control, 10 guinea pigs received a 0.1% suspension of dinitrochlorobenzene in acetone for the induction and challenge phases of the study.

C. COMPLIANCE

- A signed Quality Assurance statement was provided

C. Results

Range Finding Study: No erythema or edema was observed. Therefore, the test compound was tested in undiluted form in the main study.

Main Study: In the induction phase at 24 hours, very slight erythema was observed, but no edema, in 2 of 10 animals after the first skin application. Subsequently, very slight erythema in only 1 animal of 10 in the group was seen after induction #6, 7, 8, and 9. Each consisted of a score of 1 for erythema.

No skin sensitization reaction was observed in any animals in test group or in the naive control group.

Classification: Supplementary

The purity of urea and the percent of urea in the test substance were not indicated.