CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 20-985

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: 5-Fluorouracil Microsponge formulation, Actinic Keratosis

Reviewer Name: Barbara Hill
Division Name: Dermatologic and Dental Drug Products
HFD#: HFD-540
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NDA number: 20-985
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Information to sponsor: Yes () No (X)

Sponsor: Dermik Laboratories, Inc.
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P.O. Box 1200
Collegeville, PA 19426-0107
(610) 454-8000

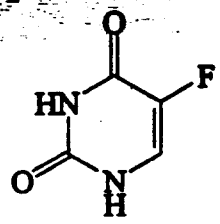
Manufacturer for drug substance:

Pharmaceutical Manufacturing Research Services, Inc.
423 Sargon Way
Horsham, PA 19044

Drug:

Code Name: DL 6025
Generic Name: Fluorouracil topical cream 0.5%, 5-Fluorouracil Microsponge® formulation 0.5%
Trade Name: ——— Cream 0.5%
Chemical Name: 2,4(1H,3H)-Pyrimidinedione, 5-Fluoro-
CAS Registry Number: 51-21-8
Molecular Formula/ Molecular Weight: $C_4H_3FN_2O_2$ / 130.08
UV Absorption: UV max (0.1 N HCl) - 265-266 nm

Structure:



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Relevant INDs/NDAs/DMFs:

1) IND ——— (5-Fluorouracil Microsponge formulation; actinic keratosis; HFD-540)
2) NDA 16-765 (Fluoroplex {Fluorouracil} 1% topical solution; actinic keratosis; HFD-540)
3) NDA 16-988 (Fluoroplex {Fluorouracil} 1% topical cream; actinic keratosis; HFD-540)
4) NDA 16-831 (Effudex {Fluorouracil} 5% topical cream and 2 and 5% topical solution; actinic keratosis; HFD-540)

Drug Class: Antimetabolite

Indication: Topical treatment of multiple actinic or solar keratoses of the face and scalp.

Clinical formulation:

Cream formulation that contains a 0.5% concentration of 5-Fluorouracil — in a microsponge polymer (an acrylate ——— .

Substance	%W/W
Purified water, USP	
Carbomer 940, NF	
Glycerin, USP	
5-Fluorouracil, USP	
Polyethylene Glycol 400, NF	
Polysorbate 80, NF	
Sorbitan Monoleate, NF	
Octyl Hydroxy Stearate, —	
Methyl Gluceth-20, —	
Stearic acid, NF	
5-Fluorouracil in Acrylates ———	
— Dimethicone in Acrylates ———	
Trolamine, — NF	
Propylene Glycol, USP	
Methylparaben, NF	
Propylparaben, NF	

Note: The 5-fluorouracil is added to the preparation as both 5-Fluorouracil, USP ——— ——— and as 5-fluorouracil in Acrylates ———

Note: The original formulation for this product contained ——— as an ——— Stability testing of Phase 3 clinical batches (containing — was unsatisfactory according to the sponsor. The sponsor proposed that this was probably due to the

interaction between — and 5-fluorouracil. A downward trend in the amount of 5-fluorouracil was observed in the stability study up to 12 months. The sponsor states that although the product remained within specifications, the 12 month regression analysis did not project a shelf life, which would be commercially acceptable. The sponsor decided to remove — from the formulation to be marketed and allow the ——— combination for the marketed formulation to consist of ——————— only.

All nonclincal toxicity studies were performed with the formulation that contained —. The removal of this ——— from the final formulation does not invalidate these studies. It will not be required of the sponsor to perform additional nonclinical toxicity studies due to this formulation change.

Dose:

The sponsor stated that the anticipated maximum clinical dose would be 2.0 g of the 0.5% 5-fluorouracil cream formulation applied once daily over the face and scalp for up to four weeks. Therefore, the maximum human daily dose would be 0.2 mg/kg/day for a 50 kg person (7.4 mg/m^2/day).

Route of administration: Topical dermal

Disclaimer: Note some material may be taken directly from sponsor's submission.

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INTRODUCTION AND DRUG HISTORY:

5-Fluorouracil has been used for over 30 years as an antineoplastic agent administered by the parental (intravenous) route. It has also been used for almost 30 years in topical formulations of 1 to 5% for the treatment of actinic keratosis (i.e., Efudex® and Fluroplex®).

5-Fluorouracil has several mechanisms of action at the molecular level. It inhibits deoxyribonucleic acid (DNA) synthesis by competitively inhibiting thymidylate synthetase. To a lesser extent, 5-fluorouracil may also interfere with ribonucleic acid (RNA) synthesis by its incorporation into RNA and by blocking uracil. These effects on DNA and RNA synthesis disrupt cell metabolism, leading to cell death. Effects are generally greater in rapidly dividing cells, which take up 5-fluorouracil more rapidly during growth and division. As a cytotoxic agent, 5-fluorouracil was first used systemically as antineoplastic therapy for a variety of tumors.

Actinic keratoses occur commonly on sun exposed skin of middle aged and older individuals, particularly fair skinned persons living in sunny regions. Actinic keratosis lesions are the result of cumulative UVB radiation and may be precursors of squamous cell carcinoma of the skin. The use of topical 5-fluorouracil for the treatment of actinic keratosis has been well documented in the medical literature. Two formulations of topical 5-fluorouracil are available in the United States (e.g., Efudex and Fluoroplex). The use of topical 5-fluorouracil for actinic keratosis has been effective but is associated with severe skin irritation, which may limit its usefulness. The current product is a new formulation of 5-fluorouracil which —— the 5-fluorouracil in a microsponge delivery system. The microsponge polymer is composed of porous acrylate (Methyl Methacrylate/ —— Glycol Dimethacrylate) —— microspheres. The sponsor hoped that less irritation would occur by incorporation of the drug into the microsponge polymer.

The microsponge technology is employed in a number of cosmetic products such as Oil of Olay Intensive Moisture Complex, Estee Lauder Face Powder and Face Wash, and Clinique Daily Eye Benefits. The microsponge system materials are listed in the CTFA Dictionary (Cosmetics, Toiletries and Fragrance Association, 7th edition, pages 26 and 2126, 1997). Recently, a New Drug Application was filed for ——— and a NDA (NDA 20-475) has been approved for a trans-retinoic acid, intended for the treatment of acne, that used the ——— process.

The nonclinical toxicology of systemically (oral, intravenous, etc) or topically administered 5-fluorouracil has been established previously in the literature. The main adverse effects noted with parenterally administered 5-fluorouracil are on the rapidly proliferating cells of the bone marrow and the gastrointestinal tract and include leukopenia, stomatitis, gastrointestinal ulceration and bleeding, and severe diarrhea.[1,2,3,4,5,6,7,8] Central neurotoxicity,[9,10,11,12] myocardial effects,[13,14] and effects on the skin,[15,16] have also occurred after parenteral administration in animals and humans. Following topical application, effects observed

[1] Gardner MLG and Plumb JA (1981) Diuranal variation in the intestinal toxicity of 5-fluorouracil in the rat. *Clin. Sci.* 61: 717-722.

[2] Harrison SD, Denine EP and Peckham JC (1978) Qualitative and quantitative toxicity of single and sequential sublethal doses of 5-fluorouracil in BDF, mice. *Cancer Treat. Rep.* 62: 533-545.

[3] Houghton JA, HoughtonPJ and Wooten RS (1979) Mechanism of induction of gastrointestinal toxicity in the mouse by 5-fluorouracil, 5-fluorouridine, and 5-fluoro-2'-deoxyuridine. *Cancer Res.* 39: 2406-2413.

[4] Linder A, Santilli D, Hodgett J and Nerlinger C (1960) Effects of 5-fluorouracil on the hematopoietic system of the mouse. *Cancer Res.* 20: 497-507.

[5] Miller E (1971) The metabolism and pharmacology of 5-fluorouracil. *J. Surg. Oncol.* 3: 309-315.

[6] Mitchel EP and Schein PS (1984) Gastrointestinal toxicity of chemotherapeutic agents. In: *Toxicity of Chemotherapy*, Grune & Stratton, Chapter 10, pp. 269-295.

[7] Litterst CL (1987) Toxicity of antineoplastic drugs, with special reference to teratogenesis, carcinogenesis, and the reproductive system. In: *Handbook of Toxicology*, TJ Haley, WO Berndt, (eds). Hemisphere Publishing Corp. Washington, Chapter 8, pp. 310-363.

[8] Dorman DC, Coddington KA and Richardson RC (1990) 5-fluorouracil toxicosis in the dog. *J. Vet. Intern. Med.* 4: 254-257.

[9] Harvey HJ, MacEwen EG and Hayes AA (1977) Neurotoxicosis assoicated with use of 5-fluorouracil in five dogs and one cat. *J. Am. Vet. Med. Assoc.* 171: 277-278.

[10] Koenig H and Patel A (1970) Biochemical basis for fluorouracil neurotoxicity. The role of krebs cycle inhibition of fluoroacetate. *Arch. Neurol.* 23: 155-160.

[11] Weiss HD, Waler MD and Wiernik PH (1974) Neurotoxicity of commonly used antineoplastic agents. *N. Engl. J. Med.* 291: 75-81.

[12] Riehl J and Brown WJ (1964) Acute cerebellar syndrome secondary to 5-fluorouracil atherapy. *Neuroloty* 14: 961-967.

[13] IRAC (1981) Monographs on the evaluation of carcinogenic risks of chemicals to humans: 5-fluorouracil. Lyon, France, World Health Organization, International Agency for Research on Cancer. 26: 217-235.

[14] Keefe DL, Roistacher N and Pierri MK (1993) Clinical cardiotoxicity of 5-fluorouracil. *J. Clin. Pharmacol.* 33: 1060-1070.

[15] Falkson G and Schulz EJ (1962) Skin changes in patients treated with 5-fluorouracil. *Br. J. Dermatol.* 24: 229-236.

[16] Vukelja SG, Bonner MW, McCollough M, Coob PW, Gaule DA, Fanucchi, PJ and Keeling JH (1991) Unusual serpentine hyperpigmentation assoicated with 5-fluorouracil. *J. Am. Acad. Dermatol.* 25: 905-908.

at the dermal administration site have included local inflammatory reactions, photosensitivity and hyperpigmentation.[17,18]

The focus of the nonclinical program for this topical formulation of 5-fluorouracil was to provide adequate nonclinical safety data for the new formulation of 5-fluorouracil. The sponsor has not conducted any studies to evaluate the carcinogencitiy, mutagenicity, or reproductive toxicity of 5-fluorouracil incorporated into the Microsponge® system. The sponsor will rely on relevant information for these categories as they pertain to the active ingredient, 5-fluorouracil, that have been derived from the literature and are included in the NDA submission. The development program initially focused on a product with a —— 5-fluorouracil concentration and a —— 5-fluorouracil cream was used in early nonclinical and Phase 1 clinical studies. Results of a phase 2 clinical dose response study indicated that a 0.5% 5-fluorouracil cream was as efficacious as the —— 5-fluorouracil formulation but caused less dermal irritation. Therefore, later nonclinical studies (including an 8 week dermal toxicity study in micropigs and a phototoxicity study in hairless mice) and phase 3 clinical studies were conducted using a 0.5% 5-fluorouracil cream formulation. This decision received approval from the division.

It is important to note that all of the formulations tested in the nonclinical toxicity studies (except the rabbit primary irritation study with the —— 5-fluorouracil —— formulation) contained the ———————— The 8 week micropig study was conducted with the same 0.5% 5-fluorouracil cream formulation for which the one year stability data showed a downward trend in 5-fluorouracil concentration approaching the limits of specification. The sponsor states that the micropig study was completed within one week of the one year stability analyses. Therefore, the study was conducted with a product that was optimal for testing any potential degradates of 5-fluorouracil that might occur in the formulation. The micropig study conducted with the 0.5% 5-fluorouracil cream that contained———————— has been determined to be acceptable since the level of 5-fluorouracil in the cream formulation was within an acceptable standard deviation based on the stability data. Since the ———————— was determined to be the cause of the degradation and was removed from the formulation of the to be marketed 0.5% 5-fluorouracil cream product, the sponsor states that there is no longer a concern with this product.

STUDIES REVIEWED WITHIN THIS SUBMISSION:

Nonclinical Pharmacology Studies:

Note: Sponsor states that no pharmacology studies were conducted with 5-fluorouracil in Microsponge® in support of this NDA.

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[17] Tsuji T, Nakagawa K and Hamada T (1984) Different effects of topically applied 5-fluorouracil on hairy and hairless mice. *Clin. Exp. Dermatol.* 9: 574-582.
[18] Physcian's Desk Reference (1999) Efudex. Medical Economics Co. Inc. New Jersey, pp. 1364-1365.

Nonclinical Pharmacokinetic Studies:

Note: Sponsor states that ADME studies can be found as appendices to nonclinical toxicology reports.

Acute Toxicology Studies (5-fluorouracil cream):

1) Acute oral toxicity in rats (DL-PC-6025-9415)

Acute Toxicology Studies (microsponge):

1) Acute oral study in rats (— Study B0306S)

Special Toxicology Studies (5-fluorouracil cream):

1) Primary dermal irritation in rabbits, —— 5-FU — 9210622)
2) Primary dermal irritation in rabbits, —— 5-FU cream (DL-PC-6025-9413)
3) Primary ocular irritation in rabbits, —— 5-FU cream (DL-PC-6025-9414)
4) Topical primary irritation pre-screening study in hairless mice, 0.5% 5-FU cream (DL-PC-6025-9718)
5) Topical phototoxicity study in hairless mice, 0.5% 5-FU cream (DL-PC-6025-9719)

Special Toxicology Studies (microsponge):

1) Dermal irritation in rabbits – abraded and intact skin (— Study B0307S)
2) Ocular irritation in rabbits (— Study B0308S)

Repeat Dose Dermal Toxicology Studies (5-fluorouracil cream):

1) 5-Day dermal range finding study in rats, —— 5-FU cream (DL-PC-6025-95)
2) 90-Day dermal toxicity study in rats, —— cream (DL-PC-6025-9511)
3) 5-Day dermal range finding study in rabbits, ——cream (DL-PC-6025-9)
4) 90-Day dermal administration study in rabbits, —— 5-FU cream (DL-PC-6025-9512)
5) 7-Day dermal pilot study in rabbits, 0.5%-5-FU cream (DL-PC-6025-9701)
6) 14-Day dermal irritation study in Yucatan micropigs (DL-PC-6025-9703)
7) 8-week dermal toxicity study in Yucatan micropigs (DL-PC-6025-9704)

Reproductive Toxicology Studies (5-fluorouracil):

Note: No reproductive toxicity studies were conducted with the drug product. The sponsor will rely on literature data for 5-fluorouracil included in the NDA submission.

Genotoxicity Studies (5-fluorouracil):

Note: No genotoxicity studies were conducted with the drug product. The sponsor will rely on literature data for 5-fluorouracil included in the NDA submission.

Genotoxicity Studies (microsponge):

1) Salmonella/mammalian-microsome plate incorporation mutagenicity assay – Ames test (— Study B0165S0
2) Ames/Salmonella plate incorporation assay on microsponge with and without fluorescent light activation (— Study B0201S)
3) Mutagenicity test on Acrylates —— in the in vivo mouse micronucleus assay —— Report No. 18897-0-4550ECD)
4) Mutagenicity test with — in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay (— Study B0373S)
5) Mutagenicity test on — measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells (— Study B0374S)
6) Mutagenicity test on —— in the CHO/HGPRT forward mutation assay (— Study B0375S)

Carcinogenicity Studies (5-fluorouracil):

Note: No carcinogenicity studies were conducted with the drug product. The sponsor will rely on literature data for 5-fluorouracil included in the NDA submission.

PHARMACOLOGY:

The mechanism of action of 5-fluorouracil has been extensively studied and is well known. 5-Fluorouracil causes death of rapidly proliferating cells, primarily through the inhibition of DNA synthesis.[19,20,21] To inhibit DNA synthesis, 5-fluorouracil must first go through biochemical activation (i.e., metabolism) to form 5-fluoro-deoxyuridine monophosphate (FdUMP). This metabolite subsequently leads to the inhibition of thymidylate synthetase, an enzyme essential for DNA synthesis.

In another metabolic pathway, 5-fluorouracil can be metabolized to 5-fluorouridine-triphosphate (FUTP). This metabolite can subsequently be incorporated into all species of RNA and affect many processes important to RNA function.[22,23] Ultimately, these effects on RNA can lead to errors in protein synthesis that may contribute to cell death.

When female CD-1 mice were treated topically with 5% 5-fluorouracil (Efudex®) either prior to or after treatment with 7,12-demethybenz[a]anthracene, it was demonstrated that 5-fluorouracil killed cycling cells in the epidermis, but not quiescent cells. Also, 5-fluorouracil suppressed the usual hyperplastic response of epidermal cells to treatment with 12-O-tetradecanoylphorbol-13-acetate, reduced the number of epidermal basal cells counted in cross-

[19] Miller E (1971) The metabolism and pharmacology of 5-fluorouracil. *J. Surg. Oncol.* 3: 309-315.
[20] Parker WB and Cheng YC (1990) Metabolism and mechanism of action of 5-fluorouracil. *Pharmacol. Ther.* 48: 381-395.
[21] Pinedo HM and Peters GFJ (1988) Fluorouracil: biochemistry and pharmacology. *J. Clinic. Oncol.* 6: 1653-1664.
[22] Parker WB and Cheng YC (1990) Metabolism and mechanism of action of 5-fluorouracil. *Pharmacol. Ther.* 48: 381-395.
[23] Pinedo HM and Peters GFJ (1988) Fluorouracil: biochemistry and pharmacology. *J. Clinic. Oncol.* 6: 1653-1664.

sections of skin and suppressed DNA synthesis. These data provided evidence that rapid proliferation of epidermal cells is an important criterion of 5-fluorouracil specificity.

PHARMACOKINETICS/TOXICOKINETICS:

Absorption:

It has been demonstrated that the percutaneous flux of 5-fluorouracil is inversely proportional to the solubility of 5-fluorouracil in the vehicle. However, increasing the concentration of 5-fluorouracil above its maximal solubility (1.65% in a propylene glycol solution) did not increase percutaneous absorption across human thigh skin or hairless mouse skin.[24]

Systemic absorption of a topical 5% formulation of 5-fluorouracil in hydrophilic petrolatum was measured in human patients with actinic keratosis using tracer amounts of fluorouracil labeled with ^{14}C in the 2 position.[25] One gram of the 5-fluorouracil formulation was applied to the face and left in place for 12 hours. Urine samples were collected and total recovery was calculated at the end of 3 days. Results showed that ~6% of the topical dose was absorbed systemically.

In another human study,[26] 5-fluorouracil in a 5% ointment was labeled with ^{14}C in the 6 position because it is more reliable for measuring absorption than the label in the 2 position. Some of the label is exhaled in the form of CO_2 after metabolism with the label in the 2 position. When the formulation was applied under an occlusive dressing to the healthy skin of five people for up to 24 hours (1.4 – 1.83 mg of 5-fluorouracil/cm^2), up to 1.1% of the administered dose was excreted in the urine. An average of 92% of the initial dose was recovered from the dressings. The extent of systemic absorption was greater in three patients with psoriasis that were treated on the thorax with a similar dose of the same 5-fluorouracil formulation. Approximately 16-22% of the administered dose was recovered in the urine of the psoriasis patients. Approximately 47-50% of the initial dose was excreted in the urine in two other patients treated with the same 5-fluorouracil administered on ulcers of the leg.

It is interesting to note that when 5-fluorouracil is used systemically as an antineoplastic agent, it is usually administered intravenously because absorption through the gastrointestinal tract is unpredictable.[27] However, absorption following intraperitoneal injection in rats is rapid

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[24] Sheretz EF and Sloan KB (1987) Penetration flux of commercial topical 5-fluorouracil preparations through hairless mouse and human skin. *In vitro Clin. Res.* [Abstract] 35: 717A.

[25] Dillaha CJ, Jansen GT, Honeycutt WM and Holt GA (1965) Further studies with topical 5-fluorouracil. *Arch. Dermatol.* 92: 410-417.

[26] Erlanger M, Martz G, Ott F, Storck H, Rieder J and Kessler S (1970) Cutaneous absorption and urinary excretion of 5-^{14}-5-fluorouracil ointment applicated in an ointment to healthy and diseased skin. *Dermatologica* 140, Suppl. 1: 7-14.

[27] Pinedo HM and Peters GFJ (1988) Fluorouracil: biochemistry and pharmacology. *J. Clinic. Oncol.* 6: 1653-1664.

with higher plasma levels obtained after administration of the Tris salt compared to the sodium salt.[28]

Distribution:

Following parental or oral administration to rodents, 5-fluorouracil is widely distributed, with larger amounts reported in bone marrow, small intestine, splenic red pulp, liver, bladder and kidney.[29,30] Selective uptake by tumor tissue has been reported but varies considerably from tumor to tumor.[31]

The distribution of 5-fluorouracil was studied in male mice over a 2 week period using whole body autoradiography after intravenous injection of ^{14}C-labeled 5-fluorouracil (1.0-1.3 mg/kg).[32] The highest concentrations were observed in the liver, kidney and bladder over the first 20 minutes. This is consistent with rapid metabolism and excretion of the drug. After four days, the highest radioactivity was in the pancreas, lymphoid tissue, intestine and bone marrow. Radioactivity continued to be found in the pancreas after 14 days.

Studies in rats, rabbits and monkeys have shown that 5-fluorouracil is not bound to plasma protein.[33,34,35]

Following a controlled intravenous injection of ^{14}C-labeled 5-fluorouracil solution into rhesus monkeys (5 ml at a rate of 1.1 ml/min), 91% of the dose was cleared from the vascular compartment within five minutes and 98% by one hour.[36] This is consistent with rapid distribution and elimination of 5-fluorouracil. Approximately 0.13% of the dose was found in the cerebrospinal fluid (CSF) and 0.17% was found in the brain during the experimental hour.

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[28] Plumb JA and Gardner MLG (1981) Differnetial toxicity and pharmacokinetics of sodium and Tris salts of 5-fluorouracil in the rat. *Clin. Sic.* 60: 707-710.
[29] IRAC (1981) Monographs on the evaluation of carcinogenic risks of chemicals to humans: 5-fluorouracil. Lyon, France, World Health Organization, International Agency for Research on Cancer. 26: 217-235.
[30] Mossa MA, Osman AM, El-sayed M, El-aaser AA, Ismael N and El-merzabani MM (1992) Distribution and toxicity of 5-fluorouracil after intraperitoneal and anal submucosal administration. *J. Pharm. Belg.* 47: 129-134.
[31] IRAC (1981) Monographs on the evaluation of carcinogenic risks of chemicals to humans: 5-fluorouracil. Lyon, France, World Health Organization, International Agency for Research on Cancer. 26: 217-235.
[32] Appelgren L-E, Hamberger B and Theve NO (1979) The distribution of ^{14}C-labelled 5-fluorouracil in mice. *Front. Gastrointest. Res.* 5: 62-70.
[33] IRAC (1981) Monographs on the evaluation of carcinogenic risks of chemicals to humans: 5-fluorouracil. Lyon, France, World Health Organization, International Agency for Research on Cancer. 26: 217-235.
[34] Yamashita S, Nadai T, Sumi M and Suda Y (1994) Possible role of serum protein binding to improve durg disposition. *Int. J. Pharmaceutics* 108: 241-247.
[35] Bouke RS, West CR, Chheda G and Tower DB (1973) Kinetics of entry and distribution of 5-fluorouracil in cerebrospinal fluid and brain following intravenous injection in a primate. *Cancer Res.* 33: 1735-1746.
[36] Bouke RS, West CR, Chheda G and Tower DB (1973) Kinetics of entry and distribution of 5-fluorouracil in cerebrospinal fluid and brain following intravenous injection in a primate. *Cancer Res.* 33: 1735-1746.

Metabolism:

The metabolic pathways of 5-fluorouracil have been established previously in the literature. [37,38,39,40] Up to 80% of the 5-fluorouracil dose is eliminated through metabolic degradation, primarily in the liver. The plasma half-life is 10-20 minutes in several species.

Both the catabolic and anabolic pathways have been described for 5-fluorouracil metabolism. Catabolism of 5-fluorouracil is analogous to that of uracil and proceeds largely in the liver to first form dihydrofluorouracil and then ultimately α-fluoro-β-alanine, urea, carbon dioxide and ammonia. During anabolism, 5-fluorouracil can be converted to 5-fluoro-deoxyuridine monophosphate, which is an important inhibitor of thymidylate synthetase, an essential enzyme of DNA synthesis. This is the primary mechanism of action of 5-fluorouracil in the treatment of various forms of cancer. Alternately, 5-fluorouracil can undergo anabolism to fluorinated uridine, which can be incorporated into RNA after phosphorylation.

The kinetics and tissue distribution of α-fluoro-β-alanine , one of the major catabolites of 5-fluorouracil was studied in rats following intravenous bolus administration of radiolabeled α-fluoro-β-alanine.[41] The major pathway of elimination of α-fluoro-β-alanine was urinary excretion with 70% of the administered dose excreted over 192 hours. Fecal excretion represented a minor elimination pathway for α-fluoro-β-alanine, with ~10% of the administered dose excreted over 192 hours. During the first 30 min, the highest levels of tissue radioactivity were found in the kidneys, liver, spleen, lungs and heart. Radioactivity was retained over longer time periods in the enterohepatic circulation, central nervous system, heart and skeletal muscle.

Excretion:

Up to 80% of a parenteral or oral dose is metabolically degraded in mice and rats, primarily in the liver, while the remainder is excreted unchanged in the urine.[42] Metabolites can be excreted in the urine or bile, or as respiratory carbon dioxide.[43,44]

Human Pharmacokinetic data obtained for ——

The sponsor conducted a 28 day pharmacokinetic study designed to evaluate the extent of systemic exposure to 5-fluorouracil from the topical application —— under conditions of

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[37] Miller E (1971) The metabolism and pharmacology of 5-fluorouracil. *J. Surg. Oncol.* 3: 309-315.
[38] IRAC (1981) Monographs on the evaluation of carcinogenic risks of chemicals to humans: 5-fluorouracil. Lyon, France, World Health Organization, International Agency for Research on Cancer. 26: 217-235.
[39] Miller E (1971) The metabolism and pharmacology of 5-fluorouracil. *J. Surg. Oncol.* 3: 309-315.
[40] Parker WB and Cheng YC (1990) Metabolism and mechanism of action of 5-fluorouracil. *Pharmacol. Ther.* 48: 381-395.
[41] Zhang R, Soong S-J, Liu T, Barnes S and Diasio RB (1992) Pharmacokinetics and tissue distribution of 2-fluoro-β-alaninie in rats: potential relevance to toxicity pattern of 5-fluorouracil. *Drug Metab. Dispos.* 20: 113-119.
[42] IRAC (1981) Monographs on the evaluation of carcinogenic risks of chemicals to humans: 5-fluorouracil. Lyon, France, World Health Organization, International Agency for Research on Cancer. 26: 217-235.
[43] Miller E (1971) The metabolism and pharmacology of 5-fluorouracil. *J. Surg. Oncol.* 3: 309-315.
[44] Zhang R, Soong S-J, Liu T, Barnes S and Diasio RB (1992) Pharmacokinetics and tissue distribution of 2-fluoro-β-alaninie in rats: potential relevance to toxicity pattern of 5-fluorouracil. *Drug Metab. Dispos.* 20: 113-119.

maximum use in the target population. The title of the study was "A pharmacokinetic multiple dose study of 5-FU in patients with actinic keratosis treated with (0.5%) topical cream" (Study DL-6025-9720). Efudex® cream was included in this study as a positive control.

Ten actinic keratosis patients were treated with 1 gm/day of ——— applied to the face and anterior bald scalp area for 28 days. Ten actinic keratosis patients were treated with 1 gm of Efudex® 5% cream twice daily applied to the face and anterior bald scalp area for 28 days. Plasma and urine samples were analyzed for the presence of 5-fluorouracil by ——— assays with lower limits of quantitation of ——— respectively.

The sponsor states that three patients receiving ——— had detectable plasma concentrations of 5-fluorouracil. The highest individual concentration values observed ranged from ——— ng/ml. A single patient had sufficient data points in order to calculate pharmacokinetic parameters in this treatment group. Following Efudex® 5.0% cream, nine patients had detectable plasma concentrations of 5-fluorouracil, with six patients having sufficient data points in order to calculate pharmacokinetic parameters. The maximum individual concentrations values ranged from ——— ng/ml. The mean pharmacokinetic parameters and urinary excretion levels are presented in the following table.

Treatment	Cmax (ng/ml)	Tmax (hrs)	AUC (ng·hr/ml)	Urinary Excretion (µg)
———(n=1)	0.77	1.00	2.80	2.74
Efudex® 5% cream (n=6)	11.49	1.03	22.39	119.83

The data suggest that 0.06% of the applied ——— dose was excreted in the urine and 0.24% of the applied Efudex® 5% cream dose was excreted in the urine. This data supports the notion that low systemic exposure is associated with topical application of ——— Confirmation of the human pharmacokinetic data for ——— provided in this NDA was received from the Clinical Pharmacology reviewer, Vaneeta Tandon.

TOXICOLOGY:

Acute Toxicology Studies (5-fluorouracil cream):

The acute LD_{50} (mean ± SE) for an acute intravenous dose of 5-fluorouracil in various species is provided in the following table.[45]

Species	LD_{50} (mg/kg)
Mouse	340 ± 17
Rat	165 ± 26
Rabbit	27 ± 5.1
Dog	31.5 ± 3.8

[45] Physcian's Desk Reference (1998) Fluorouracil injection. Medical Economics Co. Inc. New Jersey, pp. 2463-2464.

Acute Toxicology Study #1:

Acute oral toxicity in rats

Study Title: Acute oral toxicity in rats
Study No: DL-PC-6025-9415
Amendment #, Vol #: 000, 8
Conducting laboratory: [redacted]
Date of study initiation: 12/15/94
GLP compliance: Yes
QA- Report: Yes (X) No ()
Methods:

An individual dose of the undiluted test material was administered via gavage to each rat.

Dosing:

- *species/strain:* Wistar albino rats
- *#/sex/group or time point:* 5/sex/dose
- *age:* 6 – 9 weeks
- *weight:* 190 – 220 grams
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 5 g/kg — 5-Fluorouracil cream
- *route, form, volume, and infusion rate:* route = oral (gavage)

Drug, lot#, radiolabel, and % purity: [redacted]5-Fluorouracil cream – Lot# 41002-FU

Formulation/vehicle: Same as clinical formulation except with the addition of [redacted] as a [redacted]

Observations and times:

- *Mortality:* daily for 14 days
- *Clinical signs:* daily for 14 days
- *Body weights:* prior to dose administration and on day 14
- *Gross pathology:* at sacrifice; 14 days after dosing

Results:

- **Mortality** No treatment related deaths were noted in this study.
- **Clinical signs** No treatment related clinical were noted in this study.
- **Body weights** No treatment related effects on body weight were noted in this study.
- **Gross pathology** No treatment related gross pathology effects were noted in this study.

Key Study Findings:

The estimated oral LD_{50} for rats was determined to be greater than 5 g/kg of —5-fluorouracil cream.

Acute Toxicology Studies (microsponge):

Acute Toxicology Study #1:

Acute oral study in rats

Study Title:	Acute oral study in rats
Study No:	— Study B0306S
Amendment #, Vol #:	000, 13
Conducting laboratory:	
Date of study initiation:	1/17/95
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

An individual dose of the undiluted test material was administered via gavage to each rat.

Dosing:

- *species/strain:* Sprague Dawley rats
- *#/sex/group or time point:* 5/sex/dose
- *age:* not stated
- *weight:* 200 – 300 grams
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 5 g/kg — Acrylates ——
- *route, form, volume, and infusion rate:* route = oral (gavage)

Drug, lot#, radiolabel, and % purity: — Acrylates —— - Lot# — -04-10-L125B

Formulation/vehicle: Acrylate —— formulation used for the microsponge portion of the 5-fluorouracil cream; made up as a —— concentration in ——

Observations and times:

- *Mortality:* twice daily for 14 days
- *Clinical signs:* twice daily for 14 days
- *Body weights:* prior to dose administration and then weekly
- *Gross pathology:* at sacrifice; 14 days after dosing

Results:

- **Mortality** No treatment related deaths were noted in this study.
- **Clinical signs** No treatment related clinical were noted in this study.
- **Body weights** No treatment related effects on body weight were noted in this study.
- **Gross pathology** No treatment related gross pathology effects were noted in this study.

Key Study Findings:

The estimated oral LD_{50} for rats was determined to be greater than 5 g/kg of — Acrylates ——

Special Toxicology Studies (5-fluorouracil cream):

Special Toxicology Study #1:

Primary dermal irritation in rabbits, ← 5-FU —

Study Title:	Primary dermal irritation in rabbits, ——5-FU —
Study No:	9210622
Amendment #, Vol #:	000, 12
Conducting laboratory:	[redacted]
Date of study initiation:	1/11/93
GLP compliance:	No
QA- Report:	Yes () No (X)
Methods:	

Animals were clipped free of fur at the test site 24 hours prior to application of the test article. The test article was applied to the intact skin treatment site. Each test site was covered with a 6 cm square gauze patch. The patch was held in place with non-irritating tape. The entire area was covered with two layers of gauze held in place with —— tape. The skin was exposed to the test article for four hours. After the exposure period the patches and any residual test material was removed from the animal. Animals were treated once daily, 5 days/week for 2 weeks.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 3 males and 3 females
- *age:* not stated
- *weight:* 2.5 – 3.5 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.5 ml of ——5-fluorouracil —
- *route, form, volume, and infusion rate:* route = topical

Drug, lot#, radiolabel, and % purity: ——5-Fluorouracil — - Lot# DA-345-20

Formulation/vehicle: The 5-fluorouracil — formulation was not provided in the submission.

Observations and times:

- *Dermal irritation:* Animals were examined for signs of erythema, edema and any lesions at the test site 24 hours after each treatment and after each day of rest (non-treatment). Irritation was graded and scored according to the Draize technique.

Results:

- **Dermal irritation** The ——5-fluorouracil — produced very slight erythema and very slight edema at the test site during the observation period.

Key Study Findings:

The ——5-fluorouracil — was slightly irritating to rabbit skin under the conditions of this study. Since the sponsor did not provide the formulation for the 5-fluorouracil — in the submission, it is unclear how similar it is to the to be marketed 5-fluorouracil cream formulation. Therefore, the usefulness of this study is questionable.

Special Toxicology Study 2:

Primary dermal irritation in rabbits, ——5-FU cream

Study Title:	Primary dermal irritation in rabbits, —— 5-FU cream
Study No:	DL-PC-6025-9413
Amendment #, Vol #:	000, 12
Conducting laboratory:	————————————
Date of study initiation:	10/24/94
GLP compliance:	No
QA- Report:	Yes () No (X)
Methods:	

Approximately 24 hours prior to treatment, the dorsal area of each rabbit was shaved and a black permanent marker was used to mark four sites (~6 cm^2 each). Three sites for treatment and one for nontreatment. Test article was applied to the appropriate designated treatment site. Each treated area was covered with a gauze patch and the entire trunk wrapped with additional gauze and a nonabsorbent binder. The gauze and binder were held in place with surgical tape. Each rabbit wore a plastic collar during the 24 hour exposure period. Following 24 hours of exposure, the collars were removed, the compound from each site was wiped off with gauze and tap water and the sites dried with additional gauze. The untreated site was shaved with no other manipulation of the site. Observations were made for 14 days after treatment.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 3 males and 3 females
- *age:* 3 months
- *weight:* 2.13 – 2.24 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.5 ml of —5-fluorouracil cream, 5-fluorouracil vehicle cream or Efudex® cream
- *route, form, volume, and infusion rate:* route = topical

Drug, lot#, radiolabel, and % purity: — 5-Fluorouracil cream – Lot# 41002
5-Fluorouracil vehicle cream – Lot# 41004
Efudex® cream – Lot# 0685

Formulation/vehicle: The 5-fluorouracil cream formulations are the same as clinical formulation except with the addition of ——— as a ———

Observations and times:

- *Mortality:* twice daily for 14 days
- *Dermal irritation:* Skin reactions were evaluated according to the Draize method at 30 – 60 minutes after removal of the patches and daily thereafter for 14 days.
- *Body Weights:* Prior to treatment and at termination (day 14)

Results:

- **Mortality** No treatment related deaths were noted in this study.
- **Dermal irritation** Very slight erythema was observed on 3/6 animals at sites treated with Efudex® cream 30-60 minutes after patch removal. These findings resolved within 24 hours after treatment. Very slight erythema was noted on 3/6 animals at sites treated with — 5-fluorouracil cream after patch removal which lasted 1 – 5 days. Very slight epidermal scaling was also noted on 5/6 animals at sites treated with — 5-fluorouracil cream 10 days after patch removal. The scaling resolved on all but one animal by the end of the study. No dermal irritation was noted in 5-fluorouracil vehicle cream treated animals.
- **Body Weights** No treatment related effects on body weight were noted in this study.

Key Study Findings:

Both Effudex® cream and the —5-fluorouracil cream were mild dermal irritants to rabbit skin under the conditions of this study The —5-fluorouracil cream was slightly more irritating than the Effudex® cream in this study. The 5-fluorouracil vehicle cream was non-irritating to rabbit skin under the conditions of this study.

The study report states that the study was conducted in a laboratory dedicated to conducting Good Laboratory Practice studies using those practices as a guide, but their Quality Assurance Unit was not directly involved in this study. Therefore, I believe that the results of this study are acceptable for regulatory purposes.

Special Toxicology Study #3:

Primary ocular irritation in rabbits, ——5-FU cream

Study Title:	Primary ocular irritation in rabbits, —— 5-FU cream
Study No:	DL-PC-6025-9414
Amendment #, Vol #:	000, 12
Conducting laboratory:	———
Date of study initiation:	12/12/94
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

A 0.1 ml aliquot of the test material was placed into the conjuctival sac of the left eye of each rabbit with the right eye serving as the untreated control. The eyes of all animals remained unwashed for 24 hours.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 6 male rabbits
- *age:* ~3 months
- *weight:* ~2 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.1 ml —5-fluorouracil cream
- *route, form, volume, and infusion rate:* route = topical (applied to the eye)

Drug, lot#, radiolabel, and % purity: —5-Fluorouracil cream – Lot# 41002-FU

Formulation/vehicle: Same as clinical formulation except with the addition of ——— as a ———

Observations and times:

- *Eye irritation:* Observations of corneal opacity, iritis and conjunctivitis were recorded 24, 48 and 72 hours after treatment and at 4 and 7 days if irritation persisted. Irritation was graded and scored according to the Draize technique.

Results:

- **Eye irritation** The average Draize score of the unwashed test article treated eye was 3.7, 3.0, 2.0, 1.0 and 0.3 after 24 hrs, 48 hrs, 72 hrs, 4 days and 7 days, respectively.

Key Study Findings:

The —— 5-fluorouracil cream was a mild ocular irritant in rabbits under the conditions of this study.

Special Toxicology Study #4:

Topical primary irritation pre-screening study in hairless mice, 0.5% 5-FU cream

Note: The purpose of this study was to evaluate the primary irritation potential of 0.5% 5-fluorouracil cream when administered to hairless mice and to determine dose levels for the subsequent phototoxicity study.

Study Title:	Topical primary irritation pre-screening study in hairless mice, 0.5% 5-FU cream
Study No:	DL-PC-6025-9718
Amendment #, Vol #:	000, 12
Conducting laboratory:	————
Date of study initiation:	10/28/97
GLP compliance:	Yes
QA- Report:	Yes (X) No ()

Methods:

All mice were lightly anesthetized with chloral hydrate and then positioned on plastic tubing with masking tape. Test article was applied once to the dorsal area (~25 cm^2) of mice. The administration site represented ~30% of the total skin surface area of the mouse. An aluminum foil mask was placed over each animal. The mask had a single hole with a diameter of ~1.33 cm^2. The hole of the mask was placed over the mid-dorsal area. Untreated mice were anesthetized and restrained in a similar manner as treated mice. Approximately one hour after test article administration, the mice were removed from the restraint and test article was removed from the mice with water. Untreated mice under went a sham removal with water.

Dosing:

- *species/strain:* male albino hairless Crl:SKH1-*hr*BR mouse
- *#/sex/group or time point:* 3/group
- *age:* ~7 weeks
- *weight:* 28 – 33 grams
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to dosing table below

Dosing Table

Treatment	Volume (µl/25 cm²)	Dose (mg/kg)*
Untreated	0	0
5-fluorouracil vehicle cream	200	0
0.5% 5-fluorouracil cream	50	10
0.5% 5-fluorouracil cream	100	20
0.5% 5-fluorouracil cream	200	40

* - dose based on 25 gram body weight for a mouse

Drug, lot#, radiolabel, and % purity: 0.5% 5-fluorouracil cream – Lot# 970080
5-fluorouracil vehicle cream – Lot# 970051

Formulation/vehicle: Same as clinical formulation except with the addition of ——— as a ———

Observations and times:

- *Mortality:* twice daily
- *Clinical signs:* after removal of test article and on 1, 2, and 3 days after dosing
- *Local dermal signs:* after removal of test article and on 1, 2, and 3 days after dosing. The treatment sites were graded for dermal irritation using the Draize scale.
- *Body Weights:* obtained prior to dosing and on day 4

Results:

- **Mortality** No treatment related deaths were noted in this study.
- **Clinical Signs** No treatment related clinical signs were noted in this study.
- **Local dermal signs** No treatment related dermal irritation was noted in this study.
- **Body Weights** No treatment related effects on body weights were noted in this study.

Key Study Findings:

No dermal irritation was noted in hairless mice with a maximum single dose of the 0.5% 5-fluorouracil equal to 40 mg/kg (200 µl/cm^2) under the conditions of this study. Therefore, the contract lab recommended that the phototoxicity study be conducted with a volume of 200 µl/cm^2 of the 0.5% 5-fluorouracil cream and the 5-fluorouracil vehicle cream.

Special Toxicology Study #5:

Topical phototoxicity study in hairless mice, 0.5% 5-FU cream

Study Title:	Topical phototoxicity study in hairless mice, 0.5% 5-FU cream
Study No:	DL-PC-6025-9719
Amendment #, Vol #:	000, 12
Conducting laboratory:	[redacted]
Date of study initiation:	11/18/97
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

All mice were lightly anesthetized with chloral hydrate and then positioned on plastic tubing with masking tape. Test article was applied once to the dorsal area (~25 cm^2) of mice. The administration site represented ~30% of the total skin surface area of the mouse. An aluminum foil mask was placed over each animal. The mask had a single hole with a diameter of ~1.33 cm^2. The hole of the mask was placed over the mid-dorsal area. UVR exposure began ~30 minutes after completion of dose administration and the exposure period was ~30 minutes. The mice were one meter from the UVR source at the time of exposure. Following the UVR exposure, the mice were removed from the restraint and test article was removed from the mice with water. Untreated mice were anesthetized, restrained, irradiated and subjected to sham substance removal with water.

The source of irradiation was a 6.5 kw long-arc xenon water-cooled lamp that simulated mid-latitude summer sunlight. One filter ([redacted] doped glass, 1 mm thick) was used to attenuate mid-range ultraviolet light. A solar light detector was used to monitor the incident UVR and a dose of about 0.5 MedD was delivered in an exposure period of 30 minutes. One MEdD equals a UVR dose adequate to elicit a barely perceptible response in skin.

Dosing:

- *species/strain:* male albino hairless Crl:SKH1-*hr*BR mouse
- *#/sex/group or time point:* 10/group
- *age:* ~6 weeks
- *weight:* 12 – 30 grams
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to dosing table below

Dosing Table

Treatment	Volume (µl/25 cm²)	Dose (mg/kg)*
Untreated	0	0
5-fluorouracil vehicle cream	200	0
0.5% 5-fluorouracil cream	200	40
Positive control article 8-methoxypsoralen, 0.1% in methanol	200	8

* - dose based on 25 gram body weight for a mouse

Drug, lot#, radiolabel, and % purity: 0.5% 5-fluorouracil cream – Lot# 970080
5-fluorouracil vehicle cream – Lot# 970051
8-methoxypsoralen – Lot# 55H0195
Methanol – Lot# 36046

Formulation/vehicle: Same as clinical formulation except with the addition of [redacted] as a [redacted]

Observations and times:

- *Mortality:* twice daily
- *Clinical signs:* after removal of test article and on 1, 2, and 3 days after dosing
- *Local dermal signs:* after removal of test article and on 1, 2, and 3 days after dosing. The treatment sites were graded for dermal irritation using the Draize scale.
- *Body Weights:* obtained prior to dosing and on day 4

Results:

- **Mortality** No treatment related deaths were noted in this study.
- **Clinical Signs** No treatment related clinical signs were noted in this study.
- **Local dermal signs** No treatment related dermal irritation were noted in the untreated, 5-fluorouracil vehicle cream and 0.5% 5-fluorouracil cream groups. Skin responses indicative of phototoxicity occurred in all of the mice topically administered 8-methoxypsoralen (positive control). The skin responses included very mild erythema, mild edema with barely perceptible scales (flaking) at the irradiated treatment site.
- **Body Weights** No treatment related effects on body weights were noted in this study.

Key Study Findings:

The 0.5% 5-fluorouracil cream at a dose of 40 mg/kg (200 µl/25 cm^2) was not phototoxic in hairless mice under the conditions of this study.

Special Toxicology Studies (microsponge):

Special Toxicology Study 1:

Dermal irritation in rabbits – abraded and intact skin

Study Title:	Dermal irritation in rabbits – abraded and intact skin
Study No:	— Study B0307S
Amendment #, Vol #:	000, 13
Conducting laboratory:	———
Date of study initiation:	1/17/95
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

Animals were clipped free of fur at the test site 24 hours prior to application of the test article. On the right side of test site, a 21 gauge needle was used to make three 2–3 cm longitudinal abrasions through the stratum corneum to generate the abraded test site. The left side of the test site was left intact. Test material was applied to both intact and abraded skin and each test area was covered with a 2.5 cm square gauze patch. The patch was held in place with non-irritating tape. The entire area was covered with an impermeable occlusive wrapping held in place with ——— tape. The test material remained on the test site for 24 hours. After the exposure period the patches and any residual test material was removed.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 6 rabbits (sex not specified)
- *age:* ~3 months
- *weight:* ~2 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.5 g of — Acrylates ———
- *route, form, volume, and infusion rate:* route = topical

Drug, lot#, radiolabel, and % purity: — Acrylates ——— - Lot# ———-04-10-L125B

Formulation/vehicle: Acrylates ——— formulation used for the microsponge portion of the 5-fluorouracil cream (applied as the ———).

Observations and times:

- *Dermal irritation:* Skin reactions were evaluated according to the Draize method at 30 – 60 minutes after removal of the patches and after 72 hours.

Results:

- **Dermal irritation** No dermal irritation was noted at either the intact or abraded skin site of test article treated animals.

Key Study Findings:

The — Acrylates ——— was not a dermal irritant to intact or abraded rabbit skin under the conditions of this study.

Special Toxicology Study #2:

Ocular irritation in rabbits

Study Title:	Ocular irritation in rabbits
Study No:	— Study B0308S
Amendment #, Vol #:	000, 13
Conducting laboratory:	———
Date of study initiation:	1/17/95
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

A 0.03 g aliquot of the test material was placed into the conjuctival sac of the right eye of each rabbit with the left eye serving as the untreated control. The eyes of all animals remained unwashed for 24 hours.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 6 rabbits (sex not specified)
- *age:* ~3 months
- *weight:* ~2 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.03 g of — Acrylates ———
- *route, form, volume, and infusion rate:* route = topical (applied to the eye)

Drug, lot#, radiolabel, and % purity: — Acrylates ——— - Lot# ——04-10-L125B

Formulation/vehicle: Acrylates ——— formulation used for the microsponge portion of the 5-fluorouracil cream (applied as the ——).

Observations and times:

- *Eye irritation:* Observations for irritation of the cornea, iris and conjunctiva were recorded 24, 48 and 72 hours after treatment and at 4 and 7 days if irritation persisted. Irritation was graded and scored according to the Draize technique.

Results:

- **Eye irritation** Minimal conjunctival irritation was noted in the treated eye of all six animals. All signs of conjunctival irritation cleared by the third day of observation. No corneal opacities or iris irritation were noted in this study.

Key Study Findings:

The — Acrylates —— was a very mild ocular irritant in rabbits under the conditions of this study.

Repeat Dose Dermal Toxicology Studies (5-fluorouracil cream):

Repeat Dose Dermal Toxicology Study #1:

5-Day dermal range finding study in rats, ——5-FU cream

Study Title:	5-Day dermal range finding study in rats, ——5-FU cream
Study No:	DL-PC-6025-95
Amendment #, Vol #:	000, 8
Conducting laboratory:	———
Date of study initiation:	1/30/95
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

Hair from the entire dorsal trunk of each animal was removed with electric clippers. Test article was applied to the treatment site twice daily for 5 days. Animals were uncollared and the treatment site was unoccluded in this study.

Dosing:

- *species/strain:* Sprague Dawley rats
- *#/sex/group or time point:* 1/sex/group
- *age:* ~45 days
- *weight:* 194 – 232 grams
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* refer to dosing table below

- *route, form, volume, and infusion rate:* route = topical

Dosing Table

Treatment	Dose	Total Dose (mg/kg/day)
5-fluorouracil cream (low dose)	20 mg/kg, 2x/day	40
5-fluorouracil cream (mid dose)	60 mg/kg/, 2x/day	120
5-fluorouracil cream (high dose)	200 mg/kg, 2x/day	400

Drug, lot#, radiolabel, and % purity: ——5-Fluorouracil cream – Lot# 41002-FU

Formulation/vehicle: Same as clinical formulation except with the addition of ——— ——— as a ———

Observations and times:

- *Mortality:* daily
- *Local dermal signs:* The degree of erythema and edema was evaluated daily prior to the previous application and 6 hours after the last application. Skin reactions were scored according to the Draize method.
- *Body weights:* at initiation of dosing and at study termination

Results:

- **Mortality** No treatment related deaths were noted in this study.
- **Local dermal signs** The average combined erythema and edema scores after the last application for low, mid and high dose groups were 1.00, 1.17 and 1.61, respectively. The test article elicited a dose dependent mild to moderate irritation.
- **Body weights** No treatment related effects on body weight were noted in this study.

Key Study Findings:

The ——5-fluorouracil cream at doses of 40, 60 and 200 mg/kg/day was mildly to moderately irritating in rabbits after repeat dose administration under the conditions of this study (5 days; not occluded).

Repeat Dose Toxicology Study #2:

90-Day dermal toxicity study in rats, ——cream

Study Title: 90-Day dermal toxicity study in rats, —— cream
Study No: DL-PC-6025-9511

Amendment #, Vol #: 000, 8-9
Conducting laboratory: [redacted]
Date of study initiation: 3/14/95
GLP compliance: Yes
QA- Report: Yes (X) No ()
Methods:

The hair was clipped from the entire dorsal trunk of each animal 24 hours before the start of dosing and was re-clipped on an as needed basis. Test article was applied evenly over the backs of the animals to a maximum of 10% of the body surface area, beginning at the area of the spine and scapula and working posteriorly and laterally. If moderate to severe irritation occurred, the next dose was made to an adjacent site within the previously designated 10% surface area. Test article was applied twice daily for 90 days.

Dosing:

- *species/strain:* Sprague-Dawley rats
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 50 – 53 days
- *weight:* 216-326 grams males; 179-226 grams females
- *satellite groups used for toxicokinetics or recovery:* Refer to dosing table below
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Treatment	Dose	Total Dose (mg/kg/day)	Number of Study Animals	
			Males	Females
5-fluorouracil vehicle cream	60 mg/kg, 2x/day	0	10	10
[redacted] 5-fluorouracil cream	10 mg/kg, 2x/day	20	10	10
[redacted] 5-fluorouracil cream	20 mg/kg, 2x/day	40	10	10
[redacted] 5-fluorouracil cream	60 mg/kg, 2x/day	120	10	10

Drug, lot#, radiolabel, and % purity: [redacted] 5-fluorouracil cream – lot# DLC-026
5-fluorouracil vehicle cream – lot # DLC-027

Formulation/vehicle: Same as clinical formulation except with the addition of [redacted] as a [redacted]

Observations and times:

- *Clinical signs:* daily
- *Local dermal signs:* daily for the first 21 days and then weekly
- *Body weights:* weekly
- *Food consumption:* weekly

- *Hematology:* prior to terminal sacrifice (week 13)
- *Clinical chemistry:* prior to terminal sacrifice (week 13)
- *Urinalysis:* prior to terminal sacrifice (week 13)
- *Gross pathology:* at sacrifice
- *Organs weighed:* adrenals, brain, epididymides, prostate, heart, kidneys, liver, uterus, spleen, thymus, ovaries and testes
- *Histopathology:* The following organs were preserved from each animal in 10% buffered formalin: adrenals, bone with marrow, brain, epididymides, esophagus, eyes and optic nerves, Harerian glands, head, heart and aorta, large intestines (cecum, colon, rectum), small intestines (duodenum, jejunum, ileum), kidneys, lacrimal glands, liver, lungs and bronchi, cervical, mesenteric and draining lymph nodes, gross lesions, mammary glands, ovaries and oviducts, pancreas, pituitary, prostate and seminal vesicles, salivary glands, sciatic nerve, skeletal muscle, skin (treated and untreated), spleen, stomach, testes, thymus, thyroid and parathyroids, tongue and larynx, trachea, urinary bladder, uterus and vagina.

  Histological examination of the lung, liver, kidney and skin tissue was evaluated for each animal in each dose group. The remaining tissues were processed for the control and high dose group only.

Results:

- **Clinical signs** No treatment related deaths or clinical signs were noted in this study.
- **Local dermal signs** A dose-dependent increase in skin irritation after treatment with the test article was noted in this study. The degree of skin irritation ranged from moderate in the low dose group to severe in the high dose group. In all treated groups, the irritation observed after treatment was severe enough to warrant the moving of subsequent doses to an adjacent site.
- **Body weights** No treatment related effects on body weight were noted in this study.
- **Food Consumption** No treatment related effects on food consumption were noted in this study.
- **Hematology** A statistically significant increase in segmented neutrophils was noted in both male (↑2.4 fold) and female (↑2.1 fold) rats in the high dose group compared to control animals.
- **Clinical chemistry** No treatment related effects on clinical chemistry parameters were noted in this study.

- **Urinalysis** No treatment related effects on urinalysis parameters were noted in this study.
- **Organ weights** No treatment related effects on organ weights were noted in this study.
- **Gross pathology** No treatment related effects on gross pathology were noted in this study.
- **Histopathology** No treatment related histopathological effects were noted in this study.

Key Study Findings:

The major site of toxicity for the treated animals was the skin. A dose-dependent increase in skin irritation after treatment with the test article was noted in this study. The degree of skin irritation ranged from moderate in the low dose group to severe in the high dose group. In all treated groups, the irritation observed after treatment was severe enough to warrant the moving of subsequent dosages to an adjacent site. Similar effects have been documented for the marketed 5-Fluorouracil cream (i.e., Effudex®) when used clinically. In addition, a statistically significant increase in segmented neutrophils was noted in both male and female rats in the high dose group compared to control animals. Similar effects have been documented for 5-Fluorouracil when used clinically via iv administration.

Due to the skin irritation noted in all dose groups, a dermal (local) NOAEL could not be established in this study. The NOAEL for systemic effects (an increase in segmented neutrophils) was 40 mg/kg/day (240 mg/m^2/day). The NOAEL for the systemic effects in this study is ~32 times the maximum human dose (240 mg/m^2/day ÷ 7.4 mg/m^2/day).

It is unfortunate that no blood drug levels were determined in this study. It would have been useful to have included a satellite group of rats that compared the systemic absorption of the marketed 5-Fluorouracil cream product (Efudex) vs the tested drug product that contained —— 5-Fluorouracil in the microsponge delivery vehicle.

Repeat Dose Dermal Toxicology Study #3:

5-Day dermal range finding study in rabbits, ——cream

Study Title:	5-Day dermal range finding study in rabbits, —— cream
Study No:	DL-PC-6025-9
Amendment #, Vol #:	000, 8
Conducting laboratory:	——
Date of study initiation:	1/30/95
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

Hair from the entire dorsal trunk of each animal was removed with electric clippers. Test article was applied to the treatment site twice daily for 5 days. Animals were uncollared and the treatment site was unoccluded in this study.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 1/sex/group
- *age:* not stated
- *weight:* 2.01 – 2.16 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical

Dosing Table

Treatment	Dose	Total Dose (mg/kg)
– 5-fluorouracil cream (low dose)	20 mg/kg, 2x/day	40
– 5-fluorouracil cream (mid dose)	60 mg/kg/, 2x/day	120
– 5-fluorouracil cream (high dose)	200 mg/kg, 2x/day	400

Drug, lot#, radiolabel, and % purity: ——5-Fluorouracil cream – Lot# 41002-FU

Formulation/vehicle: Same as clinical formulation except with the addition of —— —— as a ——

Observations and times:

- *Mortality:* daily
- *Local dermal signs:* The degree of erythema and edema was evaluated daily prior to the previous application and 6 hours after the last application. Skin reactions were scored according to the Draize method.
- *Body weights:* at initiation of dosing and at study termination

Results:

- **Mortality** The male animal in the high dose group died prior to the day 3 morning observation. No gross changes were observed upon necropsy.

- **Local dermal signs** The average combined erythema and edema scores after the last application for low, mid and high dose groups were 1.89, 2.11 and 2.29, respectively. The test article elicited moderate irritation in all treated animals. The irritation increased slightly with increased dose.

- **Body weights** No treatment related effects on body weight were noted in this study.

Key Study Findings:

The —— 5-fluorouracil cream at doses of 40, 60 and 200 mg/kg/day was moderately irritating (with a slight increase with dose) in rabbits after repeat dose administration under the conditions of this study (5 days; not occluded). The male animal in the high dose group died prior to the day 3 morning observation. This death may have been related to treatment.

Repeat Dose Toxicology Study #4:

90-Day dermal administration study in rabbits, —— 5-FU cream

Study Title:	90-Day dermal administration study in rabbits, —— 5-FU cream
Study No:	DL-PC-6025-9512
Amendment #, Vol #:	000, 10
Conducting laboratory:	————————————
Date of study initiation:	3-21-95
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

The hair was clipped from the entire dorsal trunk of each animal 24 hours before the start of dosing and was re-clipped on an as needed basis. Test article was applied evenly over the backs of the animals to a maximum of 10% of the body surface area, beginning at the area of the spine and scapula and working posteriorly and laterally. If moderate to severe irritation occurred, the next dose was made to an adjacent site within the previously designated 10% surface area. It was anticipated that test article would be applied twice daily for 90 days.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 3 – 3.5 months
- *weight:* 2.01-2.75 kg males; 2.00-2.74 kg females
- *satellite groups used for toxicokinetics or recovery:* Refer to dosing table below
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Treatment	Dose	Total Dose (mg/kg/day)	Number of Study Animals	
			Males	Females
5-fluorouracil vehicle cream	60 mg/kg, 2x/day	0	10	10
5-fluorouracil cream	10 mg/kg, 2x/day	20	10	10
5-fluorouracil cream	20 mg/kg, 2x/day	40	10	10
5-fluorouracil cream	60 mg/kg, 2x/day	120	10	10

Drug, lot#, radiolabel, and % purity: 5-fluorouracil cream – lot# DLC-026
5-fluorouracil vehicle cream – lot # DLC-027

Formulation/vehicle: Same as clinical formulation except with the addition of [redacted] as a [redacted]

Observations and times:

- *Clinical signs:* daily
- *Local dermal signs:* daily for the first 30 days and then weekly
- *Body weights:* weekly
- *Food consumption:* weekly
- *Hematology:* planned for prior to terminal sacrifice but did not occur because study was terminated early
- *Clinical chemistry:* planned for prior to terminal sacrifice but did not occur because study was terminated early
- *Gross pathology:* at sacrifice or upon spontaneous death
- *Organs weighed:* adrenals, brain, epididymides, prostate, heart, kidneys, liver, uterus, spleen, thymus, ovaries and testes were weighed at sacrifice or upon spontaneous death
- *Histopathology:* The following organs were preserved from each animal in 10% buffered formalin at sacrifice or upon spontaneous death: adrenals, bone with marrow, brain, epididymides, esophagus, eyes and optic nerves, Harerian glands, head, heart and aorta, large intestines (cecum, colon, rectum), small intestines (duodenum, jejunum, ileum), kidneys, lacrimal glands, liver, lungs and bronchi, cervical, mesenteric and draining lymph nodes, gross lesions, mammary glands, ovaries and oviducts, pancreas, pituitary, prostate and seminal vesicles, salivary glands, sciatic nerve, skeletal muscle, skin (treated and untreated), spleen, stomach, testes, thymus, thyroid and parathyroids, tongue and larynx, trachea, urinary bladder, uterus and vagina.

  Per the sponsor's request, none of the preserved tissues either from animals that died, were sacrificed moribund or were