

CODING FORMS FOR SRC INDEXING

Microfiche No.		OTS0001088	
New Doc ID	FYI-OTS-0794-1088	Old Doc ID	
Date Produced	06/22/88	Date Received	07/26/94
		TSCA Section	FYI
Submitting Organization		DRY COLOR MFGS ASSN	
Contractor			
Document Title		INITIAL SUBMISSION: LETTER FROM DRY COLOR MFGS ASSN TO DYNAMAC CORP REGARDING RED LAKE C ANINE WITH ATTACHMENTS, DATED 06/22/88	
Chemical Category		RED LAKE C ANINE	

Effects diminished by day 4 and returned to normal by day 21. When rinsed away shortly after contact, the material did not cause corneal clouding. The irritation to the iris and conjunctive still occurred, but to a lesser degree. No permanent damage occurred although some transient severe irritation did result.

4. Ames Test Negative with and without metabolic
Salmonella Typhimurium activation

This compound did not demonstrate mutagenic potential in the tester strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 under the conditions of this assay.

5. Aquatic Toxicity LC50 170 mg/l
Bluegill

The material was found to be practically non-toxic to bluegill, a fresh water species.

6. Acute Inhalation LC50 (1 hour) > 11 mg/l
Sprague Dawley Rat

An increase in lacrimation (predominantly in males) indicated a response to exposure.

7. Octanol/Water Partition $P < 10$
Coefficient

The P value for C Amine in pH 5, pH 7 and pH 9 was found to be less than 10, indicating a very low octanol solubility.

BASF has advised DCMA that it does not consider these results or studies to be confidential.

We hope this information is of assistance to you.

Sincerely,



J. Lawrence Robinson
Executive Vice President

Enclosures

JLR/pep

File(s) searched:

File 399:CA SEARCH 1967-1988 UD-10810
(Coop. 1988 by the Amer. Chem. Socl.)

Sets selected:

Set	Items	Description
1	46	RN-88-52-9
2	22815	CARCINOGEN7
3	41678	TOXIC
4	24408	HAZARD7
5	0	RN-88-53-9 AND (CARCINOGEN7 OR TOXIC OR HAZARD7)
6	39	RN-88-51-7
7	0	RN-88-51-7 AND (S2 OR S3 OR S4)

Prints requested ('.' indicates user print cancellation) :

Date Time Description
Line# 12:08EST P011: PR 1/3/1-48
Line# 12:08EST P012: PR 8/3/1-39

Total items to be printed: 85

DIALOG FILE 300: CA SEARCH 1987-1988 US-10014 (Copr. 1988 by the Amer. Chem. Soc.)

10000876 CA: 105(8)30876R PATENT
Dyeing or printing by diazo compounds
INVENTOR(AUTHOR): Miura, Takashi
LOCATION: Japan.
ASSIGNEE: Sumitomo Chemical Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 87180189 A2 ; JP
62190259 DATE: 870820
APPLICATION: JP 8628644 (860217)
PAGES: 19 PP. CODEN: JKXKAF LANGUAGE: Japanese CLASS: C09
B-082, 12A; DOCP-001/268; C07D-251/52; C07D-151/70

10007499 CA: 108(2)7699Y PATENT
Azo printing ink pigments
INVENTOR(AUTHOR): Funatsu, Takenori; Chiba, Ateru; Kituchi,
Yoshimi
LOCATION: Japan.
ASSIGNEE: Toyo Ink Mfg. Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 87727119 A2 ; JP
8272799 DATE: 870409
APPLICATION: JP 85212600 (850927)
PAGES: 3 PP. CODEN: JKXKAF LANGUAGE: Japanese CLASS: C09B
-067/00A; C09D-011/02

10720006 CA: 107(22)20006V PATENT
Azo dye lakes
INVENTOR(AUTHOR): Zimmermann, Gesine
LOCATION: Fed. Rep. Ger.
ASSIGNEE: Bayer A.-G.
PATENT: Germany Offen. ; DE 2843512 A1 DATE: 870811
APPLICATION: DE 2843512 (851210)
PAGES: 17 PP. CODEN: GKXBX LANGUAGE: German CLASS: C09B
069/00A; C09B-029/488; C08K-005/238; C06J-001/208

10711009 CA: 107(14)11009ZV PATENT
Azo lake pigment compositions
INVENTOR(AUTHOR): Takami, Hisanori; Tanaka, Kenichi; Miura,
Toshiro
LOCATION: Japan.
ASSIGNEE: Dainichiseika Color and Chemicals Mfg. Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 8718472 A2 ; JP
8218472 DATE: 870127
APPLICATION: JP 85195195 (850716)
PAGES: 4 PP. CODEN: JKXKAF LANGUAGE: Japanese CLASS: C09B
-067/2A

10821874 CA: 108(20)21874H PATENT
Producing azo lake pigments
INVENTOR(AUTHOR): Ando, Hirohito; Takada, Zenji; Aoki,
Shigeto; Shigeta, Yuko
LOCATION: J.-pan.
ASSIGNEE: Dainippon Ink Chemical Industry Co.
PATENT: European Pat. Appl. ; EP 202906 A1 DATE: 861126
APPLICATION: EP 86309794 (860816) ; JP 85103975 (850520)

10000876 CA: 105(8)30876R PATENT
Dyeing or printing by diazo compounds
INVENTOR(AUTHOR): Miura, Takashi
LOCATION: Japan.
ASSIGNEE: Sumitomo Chemical Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 87180189 A2 ; JP
62190259 DATE: 870820
APPLICATION: JP 8628644 (860217)
PAGES: 19 PP. CODEN: JKXKAF LANGUAGE: Japanese CLASS: C09
B-082, 12A; DOCP-001/268; C07D-251/52; C07D-151/70

10000876 CA: 105(8)30876R PATENT
Azo lake pigment compositions
INVENTOR(AUTHOR): Takami, Hisanori; Tanaka, Kenichi; Takami,
Hisanori
LOCATION: Japan.
ASSIGNEE: Dainichiseika Color and Chemicals Mfg. Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 86203176 A2 ; JP
81203176 DATE: 860909
APPLICATION: JP 8541300 (850304)
PAGES: 5 PP. CODEN: JKXKAF LANGUAGE: Japanese CLASS: C09B
-067/22A; C09D-011/02B

108051784 CA: 108(8)51784B PATENT
Pigment lacquers based on 2-hydroxy-3-naphthole acid salts
INVENTOR(AUTHOR): Muzik, Ferdinand; Krstacek, Frantisek; Holc
ikova, Milena
LOCATION: Czech.
PATENT: Czechoslovakia ; CS 226132 B DATE: 860415
APPLICATION: CS 821401 (820301)
PAGES: 5 PP. CODEN: CZKXAG LANGUAGE: Czech CLASS: C09B-01
5/037

108184083 CA: 108(18)184083S JOURNAL
Development of a successful gas chromatographic method of
analyzing o-aminobenzenesulfonic acids via their sulfonyl
chlorides
AUTHOR(S): Amer. Adel; Alley, Earl G.; Pittman, Charles U.,
Jr.
LOCATION: Univ. Ind. Chem. Res. Cent., Mississippi State
Univ., Mississippi State, MS, 39762, USA
JOURNAL: J. Chromatogr. DATE: 1986 VOLUME: 382 NUMBER: 3
PAGES: 413-18 CODEN: JPCRAM ISSN: 0021-9673 LANGUAGE: Eng
lish

108184083 CA: 108(18)184083S JOURNAL
Development of a successful gas chromatographic method of
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JOURNAL: J. Chromatogr. DATE: 1986 VOLUME: 382 NUMBER: 3
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108184083 CA: 108(18)184083S JOURNAL
Development of a successful gas chromatographic method of
analyzing o-aminobenzenesulfonic acids via their sulfonyl
chlorides
AUTHOR(S): Amer. Adel; Alley, Earl G.; Pittman, Charles U.,
Jr.
LOCATION: Univ. Ind. Chem. Res. Cent., Mississippi State
Univ., Mississippi State, MS, 39762, USA
JOURNAL: J. Chromatogr. DATE: 1986 VOLUME: 382 NUMBER: 3
PAGES: 413-18 CODEN: JPCRAM ISSN: 0021-9673 LANGUAGE: Eng
lish



DIALOG File 399: CA SEARCH 1987-1988 UD-10810 (Copr. 1988 by the Amer. Chem. Soc.)

- 105001898 CA: 105(1)1988 JOURNAL
Results of microbial mutation test for forty-three
industrial chemicals
AUTHOR(S): Shimizu, Hirosuke; Suzuki, Yuji; Takemura,
Mozumi; Goto, Sumio; Matsushita, Hidetsuru
LOCATION: Sch. Med., Jikei Univ., Tokyo, Japan, 105
JOURNAL: Sengyo Igaku DATE: 1985 VOLUME: 27 NUMBER: 6
PAGES: 400-19 CODEN: SAIGBL ISSN: 0047-1879 LANGUAGE: Eng
lish
- 104234931 CA: 104(26)234931b PATENT
Pigment toners with improved transparency
INVENTOR(AUTHOR): Matrick, Howard
LOCATION: USA
ASSIGNEE: du Pont de Nemours, E. I., and Co.
PATENT: United States; US 4561899 A DATE: 851231
APPLICATION: US 673925 (841121)
PAGES: 6 pp. CODEN: USXXAM LANGUAGE: English CLASS: 10619
3000P: COBL-001/08A
- 107012220 CA: 107(12)12220e JOURNAL
Studies in lanthanide lens complexes with some substituted
sulfonic acids
AUTHOR(S): Manikade, A. S.; Khorragade, B. G.; Narwade, M.
L. LOCATION: Dep. Chem., Gov. Vidarbha Mahavidyalaya, Amravati,
444 604, India
JOURNAL: J. Indian Chem. Soc. DATE: 1984 VOLUME: 61
NUMBER: 10 PAGES: 870-1 CODEN: JICSAH ISSN: 0019-4522
LANGUAGE: English
- 102150915 CA: 102(13)150915r PATENT
Azo pigments
LOCATION: Japan
ASSIGNEE: Toyo Ink Mfg. Co., Ltd.
PATENT: Japan Kokai Tokyo Koho; JP 84191762 A2; JP
59191762 DATE: 841030
APPLICATION: JP 8365228 (830415)
PAGES: 6 pp. CODEN: UKXXAF LANGUAGE: Japanese CLASS: C09B
-041/00
- 100073778 CA: 100(10)73778a JOURNAL
Analysis of subsidiary colors in Red No. 204 and its
purification. Development of non-allergenic Red No. 204
AUTHOR(S): Negaruma, Masayuki; Onitsc, Yutaka; Kataumura,
Yoshio; Matsuoka, Masahiro; Morikawa, Yoshihiro; Tanaka,
Muneo; Mitsu, Takeo
LOCATION: Japan
JOURNAL: J. SCCJ DATE: 1983 VOLUME: 17 NUMBER: 1 PAGES:
27-34 CODEN: JDSQUO LANGUAGE: Japanese
- 100023428 CA: 100(8)20428d JOURNAL
Analysis of subsidiary colors in B & C Red No. 8 and its
purification. Development of nonfluorene D & C Red No. 8
AUTHOR(S): Negaruma, Masayuki; Onitsc, Yutaka; Kataumura,
Yoshio; Matsuoka, Masahiro; Morikawa, Yoshihiro; Tanaka,
Muneo; Mitsu, Takeo
LOCATION: Shuendo Lab., Yokonaka, Japan, 229
JOURNAL: J. Soc. Cosmet. Chem. DATE: 1983 VOLUME: 24
NUMBER: 6 PAGES: 273-84 CODEN: USCCAB ISSN: C087-8822
LANGUAGE: English
- 89076977 CA: 89(8)76977v PATENT
8-Chloro-3-toluidine-4-sulfonic acid
INVENTOR(AUTHOR): Paperfuh, Theodor; Berthold, Rüdiger
LOCATION: Fed. Rep. Ger.
ASSIGNEE: Hoechst A.-G.
PATENT: Germany Offen.; DE 3141288 A1 DATE: 890428
APPLICATION: DE 3141288 (811017)
PAGES: 10 pp. CODEN: GWXXBX LANGUAGE: German CLASS: C07C-
143/83; C07C-139/00
- 89080219 CA: 89(11)80219u JOURNAL
Chloro-substituted derivatives of
dibenzotetrahydrofuranthiatrialvalena
AUTHOR(S): Kiyuev, V. N.; Korzhnevalii, A. B.; Salkina, L.
B. LOCATION: Ivenov, Khim.-Tekhnol. Inst., Ivanovo, USSR
JOURNAL: Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol.
DATE: 1982 VOLUME: 25 NUMBER: 10 PAGES: 1178-8 CODEN: IV
UKAR ISSN: 0579-2951 LANGUAGE: Russian
- 88053403 CA: 88(7)83403p PATENT
Aminoarylsulfonic ester
INVENTOR(AUTHOR): Emda, Herbert; Blank, Heinz Ulrich; Schwab,
G. Peter
LOCATION: Fed. Rep. Ger.
ASSIGNEE: Bayer A.-G.
PATENT: Germany Offen.; DE 3114830 A1 DATE: 821104
APPLICATION: DE 3114830 (810411)
PAGES: 34 pp. CODEN: GWXXBX LANGUAGE: German CLASS: C07C-
139/00; C07C-143/58; C07C-143/60; C07C-143/63; C07C-143/64
- 88053402 CA: 88(7)83402n PATENT
Aromatic aminosulfonic acids
INVENTOR(AUTHOR): Emda, Herbert; Blank, Ulrich; Schwab,
Peter
LOCATION: Fed. Rep. Ger.
ASSIGNEE: Bayer A.-G.
PATENT: Germany Offen.; DE 3114829 A1 DATE: 821104
APPLICATION: DE 3114829 (810411)
PAGES: 27 pp. CODEN: GWXXBX LANGUAGE: German CLASS: C07C-
139/00 (cont. next page)

01ALOO File 008: CA SEARCH 1987-1988 UP-10810 (Copr. 1988 by the Amer. Chem. Soc.)

199/00: C07C-143/80: C07C-143/83

97080478 CA: 87(7)88478V PATENT
2-Chloro-3-aminotoluene-4-sulfonic acid (C acid)
LOCATION: Japan.

ASSIGNEE: Dainippon Ink and Chemicals, Inc.; Kawamura
Physical and Chemical Research Institute
PATEM: Japan Kokai Tokkyo Koho ; JP 8221982 A2 DATE: 82020

4 APPLICATION: JP 8093819 (800711)
PAGES: 7 PP. CODEN: JKKXAF LANGUAGE: Japanese CLASS: C07C
-143/83

97080478 CA: 87(1)8878N PATENT
2-Chloro-3-aminotoluene-4-sulfonic acid (C acid)
LOCATION: Japan.

ASSIGNEE: Dainippon Ink and Chemicals, Inc.; Kawamura
Physical and Chemical Research Institute
PATEM: Japan Kokai Tokkyo Koho ; JP 8221983 A2 DATE: 82020

4 APPLICATION: JP 8093820 (800711)
PAGES: 7 PP. CODEN: JKKXAF LANGUAGE: Japanese CLASS: C07C
-143/83; 8010-023/40

98217488 CA: 86(28)217488Y PATENT
2-Chloro-3-(and 6)-aminotoluene-4-sulfonic acids
LOCATION: Japan.

ASSIGNEE: Dainippon Ink and Chemicals, Inc.
PATEM: Japan Kokai Tokkyo Koho ; JP 8214971 A2 DATE: 82012

8 APPLICATION: JP 8089183 (800702)
PAGES: 7 PP. CODEN: JKKXAF LANGUAGE: Japanese CLASS: C07C
-143/88; 8010-031/02

98100109 CA: 88(12)108108T JOURNAL
Retention behavior of chromatic sulfonic acids in ion-pair
reversed-phase column liquid chromatography

AUTHOR(S): Prandi, C.; Venturini, T.
LOCATION: Cent. Ric. Mater. Coloranti, Azienda Colori Naz.
Affini, S.P.A. Montedison, Milan, Italy
JOURNAL: J. Chromatogr. Sci. DATE: 1981 VOLUME: 19
NUMBER: 6 PAGES: 208-14 CODEN: JCHSEZ ISSN: 0021-9688
LANGUAGE: English

94193701 CA: 84(24)193701W PATENT
Reactive dyes for dyeing cellulose fibers and
cellulose textiles

INVENTOR(AUTHOR): Omura, Takeshi; Tezuka, Yasuo; Sunami,
Masaki
LOCATION: Japan.
ASSIGNEE: Sunitoko Chemical Co., Ltd.
PATEM: EUROPEAN PAT. EP 22269 DATE: 810114

0160-40

APPLICATION: Japan JP 7988249 DATE: 790708
PAGES: 31 PP. CODEN: EPXJOW LANGUAGE: English CLASS: C08B
-C82/99; C09B-062/513; C09B-062/483; D06P-003/06;

93208139 CA: 93(22)208139F PATENT
AZO pigments
INVENTOR(AUTHOR): Von, Inaiah
LOCATION: USA

ASSIGNEE: American Cyanamid Co.
PATEM: United States US 4217273 DATE: 800812
APPLICATION: United States US 1547 DATE: 790108
PAGES: 4 PP. CODEN: USAKAM LANGUAGE: English CLASS: 26020
2000; C09B-029/04;

93073787 CA: 93(8)73787M PATENT

Dyeing of thermoplastic materials
INVENTOR(AUTHOR): Guenther, Paul; Henning, Georg
LOCATION: Fed. Rep. Ger.
ASSIGNEE: BASF A.-G.

PATEM: Germany Offen. DE 2845073 DATE: 800808
APPLICATION: Germany DE 2845073 DATE: 781017
PAGES: 12 PP. CODEN: GUKXBX LANGUAGE: German CLASS: C08J-
002/20; C08K-005/00;

93073781 CA: 93(8)73781U PATENT

Calcium lakes with high brilliance
INVENTOR(AUTHOR): Hennrich, Georg; Guenther, Paul
LOCATION: Fed. Rep. Ger.
ASSIGNEE: BASF A.-G.

PATEM: France Demande FR 2422926 DATE: 800229
APPLICATION: Germany DE 2834028 DATE: 780803
PAGES: 21 PP. CODEN: FRXJEL LANGUAGE: French CLASS: C08B-
029/28; C09B-043/00; C09B-093/00; C09D-017/00;

92198110 CA: 92(21)198110B PATENT

8-Chloro-3-toluidine-4-sulfonic acid
INVENTOR(AUTHOR): Kondo, Atsushi; Otsuo, Kikuo; Nishimura
Shinji
LOCATION: Japan.

ASSIGNEE: Kodogyo Chemical Co., Ltd.
PATEM: Japan Kokai Tokkyo Koho JP 78191845 DATE: 791129
APPLICATION: Japan JP 7853245 DATE: 780518
PAGES: 3 PP. CODEN: JKKXAF LANGUAGE: Japanese CLASS: C07C
-143/83;

92198228 CA: 92(22)198228N PATENT

Calcium lake with good covering power, high pure tone
brilliance, and improved fastness to light
INVENTOR(AUTHOR): Henning, Georg; Guenther, Paul
LOCATION: U.S. Rep. Ger.

(cont. next page)

DIALOG FILE 308: CA SEARCH 1907-1988 UD-10810 (Copr. 1988 by the Amer. Chem. Soc.)

ASSIGNEE: BASF A.-G.
 PATENT: Germany Offen. DE 2834028 DATE: 800221
 APPLICATION: Germany DE 2834028 DATE: 780803
 PAGES: 26 pp. CODEN: GWXXBX LANGUAGE: German CLASS: C09B-043/00; C09B-029/10; C09B-063/00; C09D-017/00;

9118748 CA: 91(18)157488 PATENT
 2-Chloro-5-aminothiolum-4-sulfonic acid and its salts
 INVENTOR(AUTHOR): Takizawa, Hidemitsu; Fukuda, Tetsuo, Imachi, Yushimi
 LOCATION: Japan.
 ASSIGNEE: Kawaken Fine Chemicals Co., Ltd.; Dainichiseika Color and Chemicals Mfg. Co., Ltd.
 PATENT: Japan Kokai Tokkyo Koho JP 7904549 DATE: 790705
 APPLICATION: Japan JP 77149208 DATE: 771214
 PAGES: 8 pp. CODEN: JKKXAF LANGUAGE: Japanese CLASS: C07C-143/83; B01J-025/00;

9020284 CA: 90(25)202846 PATENT
 6-Chloro-3-palno(2)luenesulfonic acid-(4)
 INVENTOR(AUTHOR): Tanaka, Tsuneo; Ehashi, Shigeoyuki; Kurata, Ryuichiro
 LOCATION: Japan
 ASSIGNEE: Toyo Ink Mfg. Co., Ltd.
 PATENT: Japan Kokai Tokkyo Koho JP 7919935 DATE: 790215
 APPLICATION: Japan JP 7781973 DATE: 770711
 PAGES: 4 pp. CODEN: JKKXAF CLASS: C07C-143/83;

9020283 CA: 90(25)202837 PATENT
 6-Chloro-3-palno(2)luenesulfonic acid-(4)
 INVENTOR(AUTHOR): Tanaka, Tsuneo; Ehashi, Shigeoyuki; Kurata, Ryuichiro; Nakayama, Yoichi
 LOCATION: Japan
 ASSIGNEE: Toyo Ink Mfg. Co., Ltd.
 PATENT: Japan Kokai Tokkyo Koho JP 7919935 DATE: 790215
 APPLICATION: Japan JP 7781973 DATE: 770711
 PAGES: 4 pp. CODEN: JKKXAF CLASS: C07C-143/83;

8818040 CA: 88(25)180404X PATENT
 2-Acylamino-5-chlorobenzenesulfonic acids
 INVENTOR(AUTHOR): Fuchs, Otto; Mees, Bernhard; Berthold, Ruediger; Lange, Siegfried
 LOCATION: Ger.
 ASSIGNEE: Hoechst A.-G.
 PATENT: Germany Offen. DE 2639668 DATE: 780309
 APPLICATION: Germany DE 2639668 DATE: 780903
 PAGES: 11 pp. CODEN: GWXXBX CLASS: C07C-143/87;

88051970 CA: 88(8)181970R PATENT
 Telluro-sulfon-2 acid derivatives
 INVENTOR(AUTHOR): Jenkins, Harry Lee; Smelser, Gene Clark; 4 Ford, David Wayne

LOCATION: USA
 ASSIGNEE: Chemtron Corp.
 PATENT: Germany Offen. DE 270754 DATE: 770829
 APPLICATION: United States US 170999 DATE: 760928
 PAGES: 18 pp. CODEN: GWXXBX CLASS: C07C-143/88;

8718189 CA: 87(18)181849F PATENT
 2-Amino-6-chloro-4-methylbenzenesulfonic acid
 INVENTOR(AUTHOR): Noguchi, Kazuo; Tetsuo, Tetsuo; Mitetsuymen, S. Shunichi
 LOCATION: Japan
 ASSIGNEE: Dainichiseika Color and Chemicals Mfg. Co., Ltd.
 PATENT: Japan Kokai Tokkyo Koho JP 778248 DATE: 770829
 APPLICATION: Japan JP 75140624 DATE: 751126
 PAGES: 8 pp. CODEN: JKKXAF CLASS: C07C-143/83;

8711787 CA: 87(18)1178784 PATENT
 2-Chloro-6-toluidino-4-sulfonic acid
 INVENTOR(AUTHOR): Noguchi, Kazuo; Fukuda, Tetsuo; Mitetsuymen, S. Shunichi
 LOCATION: Japan
 ASSIGNEE: Dainichiseika Color and Chemicals Mfg. Co., Ltd.
 PATENT: Japan Kokai Tokkyo Koho JP 776229 DATE: 770829
 APPLICATION: Japan JP 75128712 DATE: 751113
 PAGES: 5 pp. CODEN: JKKXAF CLASS: C07C-143/83;

8817884 CA: 88(24)17884F JOURNAL
 Activated sludge degradability of organic substances in the waste water of the Kashima Petroleum and Petrochemical Industrial Complex in Japan
 AUTHOR(S): Mizui, S.; Murakami, T.; Sasaki, T.; Hirose, Y.; Iguma, Y.
 LOCATION: Off. Constr. Kashima Ind. Complex, Fukushima, Japan
 JOURNAL: P 99. Water Technol. DATE: 1978 VOLUME: 7
 NUMBER: 2-3-4 PAGES: 845-89 CODEN: POWT12 LANGUAGE: Engli sh

8604471 CA: 86(8)44719 PATENT
 Fluorescent whitening agent for synthetic polyamide fibers
 INVENTOR(AUTHOR): Moriguchi, Shojiro; Yoshida, Katsuhiko
 LOCATION: Japan
 ASSIGNEE: Dainichiseika Color and Chemicals Mfg. Co., Ltd.
 PATENT: Japan Tokkyo Koho JP 762867 DATE: 760721
 APPLICATION: Japan JP 70114118 DATE: 701221
 PAGES: 13 pp. CODEN: JAKXAD CLASS: D06L-003/12;

PRINTS

User: 001000 1 March 1978 PR 1/3/1-16

PAGE: 20 of 4

DIABLO File 200: CA SEARCH 1007-1000 US-10010 (Cap. r. 1000 by the Amer. Chem. Soc.)

00010046 CA: 06(4)100409 PATENT

Easily dispersible azo pigments

INVENTOR(AUTHOR): Rejzelski, Marian; Korzeniowska, Julitta; Siewka, Wieslaw

LOCATION: Pol.

ASSIGNEE: Miejskie Zaklady Przemyslu Barwnikow

PATENT: Poland PL 72003 DATE: 740020

APPLICATION: Poland PL 183476 DATE: 720214

PAGES: 2 pp. CODEN: POKX57 CLASS: C00B;

00000000 CA: 06(2)000000 PATENT

Azo dyes converted into a lake

INVENTOR(AUTHOR): Lendler, Josef; Moorfeld, Erhard

LOCATION: Ger.

ASSIGNEE: Hoechst A.-G.

PATENT: Germany DE 2502791 DATE: 760725

APPLICATION: Germany DE 2502791 DATE: 750120

PAGES: 9 pp. CODEN: G0XKXW CLASS: C00B-02B/00;

04100400 CA: 04(23)100400K PATENT

2-Amino-8-thiolo-6--s-thylohexamethylen-10-sulfid

INVENTOR(AUTHOR): Kinoshita, Shochi; Oyama, Masaji

LOCATION: Japan

ASSIGNEE: Dainippen Ink and Chemicals, Inc.; Kowemu-Rikagaku Kenkyukai

PATENT: Japan Kokai Tokkyo Koho JP 7608242 DATE: 760123

APPLICATION: Japan JP 7480966 DATE: 740718

PAGES: 9 pp. CODEN: JUKXAF CLASS: C07C

01010040 CA: 01(3)10040J PATENT

Amidobenzene

INVENTOR(AUTHOR): Muehmerle, Kurt; Albers, Arnold; Mille, Ernst; Meiss, Horst

ASSIGNEE: Farbwerke Hoechst A.-G.

PATENT: Germany Offen. DE 2240040 DATE: 740214

APPLICATION: Germany DE 2240040 DATE: 720819

PAGES: 24 pp. CODEN: G0XKXW CLASS: C 07C

00070100 CA: 00(10)70100K JOURNAL

Gas chromatography of aminobenzene sulfonic acids by forming

volatile sulfonyl and phosphoramidic chloride derivatives

AUTHOR(S): Parsons, J. S.

LOCATION: Chem. Res. Div., Am. Cyanamid Co., Bound Brook, N. J.

JOURNAL: J. Chromatogr. Sci., DATE: 1973 VOLUME: 11

NUMBER: 12 PAGES: 859-60 CODEN: JCH50Z LANGUAGE: English

79010020 CA: 78(10)0020Y PATENT

Azo pigment toners

INVENTOR(AUTHOR): Price, David; Hamilton, Alexander; Bradley, Gordon Frank

ASSIGNEE: Ciba-Geigy (UK) Ltd.

PATENT: Britain GB 191947 DATE: 730411
APPLICATION: Britain GB 363170 DATE: 711008
PAGES: 6 pp. CODEN: G0XKXA CLASS: C 00B

78110000 CA: 78(20)110000J PATENT
Crystallizable polyolefin-polyamide thermoplastic materials
INVENTOR(AUTHOR): Eichers, Ursula; Heilmann, Otto; Meyer, Heinz; Heilmann, Rambusch, Konrad; Rosebach, Manfred
ASSIGNEE: Chemische Werke Huls A.-G.
PATENT: Germany Offen. DE 2002489 DATE: 710729
APPLICATION: Germany DATE: 700121
PAGES: 17 pp. CODEN: G0XKXB CLASS: C 00K

73022300 CA: 73(0)23200Y PATENT
Retarding agent for epoxide resins
INVENTOR(AUTHOR): Lieske, Edgar; Meintrich, Erwin
ASSIGNEE: Henkel und Cie. G.m.b.H.
PATENT: Germany Offen. DE 1904934 DATE: 691120
APPLICATION: Switzerland DATE: 690479
PAGES: 10 pp. CODEN: G0XKXB CLASS: C 00C

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PIALOG

DIALOG P. 388: CA SEARCH 1987-1988 UD=10810 (Cmpr. 1988 by the Amer. Chem. Soc.)

PAGE: 1 of 2

100023331 CA: 108(4)22:221W PATENT
 Manufacture of azo pigments with controlled particle size
 INVENTOR(AUTHOR): Hotta, Seishi; Okabe, Hiromichi
 LOCATION: Japan.
 ASSIGNEE: Sumitomo Chemical Co., Ltd.
 PATENT: Japan Kokai Tokkyo Koho ; JP 8716986C A2 ; JP
 62169862 DATE: 870727
 APPLICATION: JP 8811205 (880121)
 PAGES: 5 PP. CODEN: JKKXAF LANGUAGE: Japanese CLASS: C:JPR
 -067/22A

107119883 CA: 107(14)11989V PATENT
 Azo lake pigment compositions
 INVENTOR(AUTHOR): Takami, Hisanori; Tanaka, Kenichi; Miura,
 Toshiko
 LOCATION: Japan.
 ASSIGNEE: Dainichiseika Color and Chemicals Mfg. Co., Ltd.
 PATENT: Japan Kokai Tokkyo Koho ; JP 8718472 A2 ; JP
 6218472 DATE: 870727
 APPLICATION: JP 85155155 (850716)
 PAGES: 4 PP. CODEN: JKKXAF LANGUAGE: Japanese CLASS: C:088
 -067/24A

107080630 CA: 107(3)0030T JOURNAL
 Metal salt size pigments derived from
 3-hydroxy-2-naphthohydroxamic acid
 AUTHOR(S): Christie, R. M.; Moss, S.
 LOCATION: Dep Technol., Scgtt. Coll., Text., Galashiels, UK.
 JOURNAL: Dyes Pigm. DATE: 1987 VOLUME: 8 NUMBER: 3
 PAGES: 211-24 CODEN: DYPIDX ISSN: 0143-7208 LANGUAGE: Eng
 1158

108178118 CA: 108(22)178118H PATENT
 Azo lake pigment compositions
 INVENTOR(AUTHOR): Takami, Hisanori; Aoki, Kazutaka; Iwata,
 Hiromichi
 LOCATION: Japan.
 ASSIGNEE: Dainichiseika Color and Chemicals Mfg. Co., Ltd.
 PATENT: Japan Kokai Tokkyo Koho ; JP 8827221 A2 ; JP
 6127221 DATE: 881202
 APPLICATION: JP 85113297 (850128)
 PAGES: 5 PP. CODEN: JKKXAF LANGUAGE: Japanese CLASS: C:088
 -063/00A; C:090-31/02

108178089 CA: 108(22)178089J PATENT
 Metal complex azo dye
 INVENTOR(AUTHOR): Niculescu, Elena C.; Stancescu, Lidia; Popo
 vic, Nicolae; Nanea, Stefan; Biliu, Ion; Rotaru, Lidia; Popesc
 u, Corneli; Mihaescu, Ana
 LOCATION: Rom.
 ASSIGNEE: Combinatu' Chimic, Giurgiu
 PATENT: Romania ; RO 86630 B1 DATE: 860331

108024928 CA: 108(9)24928H PATENT
 Azo pigment lakes
 INVENTOR(AUTHOR): Hotta, Seishi; Kuriyama, Takayoshi
 LOCATION: Japan.
 ASSIGNEE: Sumika Color Co., Ltd.
 PATENT: Japan Kokai Tokkyo Koho ; JP 86123666 A2 ; JP
 61123668 DATE: 860611
 APPLICATION: JP 84244061 (841113)
 PAGES: 4 PP. CODEN: JKKXAF LANGUAGE: Japanese CLASS: C:088
 -031/00A

108184603 CA: 108(18)184603S JOURNAL
 Development of a successful gas chromatographic method of
 analyzing 8-aminobenzene-sulfonic acids via their sulfonyl
 chlorides
 AUTHOR(S): Amer, Adel; Alley, Earl G.; Pittman, Charles U.,
 Jr.
 LOCATION: Univ Ind, Chem, Res, Cent., Mississipp, State
 Univ., Mississippi State, MS, 39762, USA
 JOURNAL: J. Chromatogr. DATE: 1986 VOLUME: 362 NUMBER: 3
 PAGES: 413-18 CODEN: JOCRAM ISSN: 0021-9673 LANGUAGE: Eng
 1168

108081714 CA: 108(8)81704H PATENT
 Pigment lacquers based on 3-hydroxy-2-naphthoic acid salts
 INVENTOR(AUTHOR): Muzik, Ferdinand; Krizek, Frantisek; Hald
 ikova, Milena
 LOCATION: Czech.
 PATENT: Czechoslovakie ; CS 226132 B DATE: 860418
 APPLICATION: CS 821401 (820301)
 PAGES: 5 PP. CODEN: CZXXAB LANGUAGE: Czech CLASS: C:088-03
 5/037

108087358 CA: 108(10)887358F PATENT
 Azo lake pigment compositions
 INVENTOR(AUTHOR): Tonara, Kazuo; Tanaka, Kenichi; Takami,
 Hisanori
 LOCATION: Japan.
 ASSIGNEE: Dainichiseika Color and Chemicals Mfg. Co., Ltd.
 PATENT: Japan Kokai Tokkyo Koho ; JP 86203176 A2 ; JP
 61203176 DATE: 860109
 APPLICATION: JP 841300 (840304)
 PAGES: 6 PP. CODEN: JKKXAF LANGUAGE: Japanese CLASS: C:088
 -067/22A; C:790-011/02B

DIALOC File 300: CA SEARCH 1987-1988 US-10010 (Cmpr. 1988 by the Amer. Chem. Soc.)

- 100101672 CA: 100(20)101672w PATENT
Azo pigment compositions
LOCATION: Japan.
ASSIGNEE: Teyo Ink Mfg. Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 85124957 A2 ; JP
80124957 DATE: 850733
APPLICATION: JP 82231954 (831209)
PAGES: 7 pp. CODEN: JMKXAF LANGUAGE: Japanese CLASS: C09B
-029/15A; C09B-011/02B
- 100000000 CA: 100(12)000000 PATENT
Reactive disease eyes for cellulose or cellulose-containing
fibers
LOCATION: Japan.
ASSIGNEE: Nippon Kayaku Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 8590208 A1 ; JP
800208 DATE: 850221
APPLICATION: JP 83188286 (831024)
PAGES: 9 pp. CODEN: JMKXAF LANGUAGE: Japanese CLASS: C09B
-002/09A; D09F-002/06B
- 102100023 CA: 102(12)100023w PATENT
Electrophotographic plates
LOCATION: Japan.
ASSIGNEE: Dainippon Ink and Chemicals, Inc.
PATENT: Japan Kokai Tokkyo Koho ; JP 84174849 A2 ; JP
89174849 DATE: 841002
APPLICATION: JP 8247872 (820224)
PAGES: 29 pp. CODEN: JMKXAF LANGUAGE: Japanese CLASS: G03
B-006/19; G03G-006/06; G03G-005/07
- 100000000 CA: 100(8)000000 PATENT
Pyrazolone azo pigments
INVENTOR(AUTHOR): Herming, Georg
LOCATION: Fed. Rep. Ger.
ASSIGNEE: BASF A.-G.
PATENT: Germany Offen. ; DE 2318073 A1 DATE: 141122
APPLICATION: DE 2318073 (820518)
PAGES: 8 pp. CODEN: GYXBXK LANGUAGE: German CLASS: C09B-0
29/50; C09K-005/24; C09B-017/00
- 101112421 CA: 101(14)112421y PATENT
Azo compounds
INVENTOR(AUTHOR): Merris, David James; Mc Mahon, Barry John;
Miller, Horace Vernon
LOCATION: Swiss.
ASSIGNEE: Ciba-Geigy A.-G.
PATENT: Germany Offen. ; DE 3226247 A1 DATE: 140412
APPLICATION: DE 3226247 (821008) (821008)
PAGES: 23 pp. CODEN: GYXBXK LANGUAGE: German CLASS: C09B-
041/00; C09B-049/01; C09B-087/20; C09B-087/46; C19D-017/00; C0
7C-07/00
- 100000510 CA: 100(2)00510w PATENT
2-Chloro-4-aminotoluene-S-sulfonic acid
INVENTOR(AUTHOR): Viadutiu, Liene Maria; Atanasiu-Merces,
Carmen Violeta Maria; Spilledis, Apostol; Dobrescu, Dumitru; A
Idoa, Violeta Ion
LOCATION: Rom.
ASSIGNEE: Combinatul Chimic, Giurgiu
PATENT: Romania ; RO 76842 B DATE: 810820
APPLICATION: RO 84821 (780728)
PAGES: 4 pp. CODEN: RUXKX3 LANGUAGE: Romanian CLASS: C07C
-142/10
- 89072100 CA: 89(10)72100a PATENT
Reactive disease dyes
LOCATION: Japan.
ASSIGNEE: Sumitomo Chemical Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 8908764 A2 ; JP
8908764 DATE: 830118
APPLICATION: JP 81107320 (810708)
PAGES: 4 pp. CODEN: JMKXAF LANGUAGE: Japanese CLASS: C09B
-062/513
- 89029800 CA: 89(8)29800a PATENT
Azo pigments
INVENTOR(AUTHOR): Ueno, Ryuzo; Tsuchiya, Hiroaki; Itoh,
Shigeru
LOCATION: Japan.
ASSIGNEE: Kabushiki Kaisha Ueno Suisyaku Dyo Kenkyujo
PATENT: European Pat. Appl. ; EP 74117 A2 DATE: 830216
APPLICATION: EP 82108231 (820807) (JP 81/140368 (810808)
PAGES: 8 pp. CODEN: EPXDXM LANGUAGE: English CLASS: C09B-
029/15; C09B-063/00; C09B-087/00 DESIGNATED COUNTRIES: BE; CH
; DE; FR; GB; IT; LI; NL
- 89022800 CA: 89(4)29800r PATENT
Disease reactive dyes
LOCATION: Japan.
ASSIGNEE: Sumitomo Chemical Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 8302354 A2 ; JP
8902354 DATE: 830107
APPLICATION: JP 81102544 (810830)
PAGES: 3 pp. CODEN: JMKXAF LANGUAGE: Japanese CLASS: C09B
-062/513
- 88197770 CA: 88(23)197770k PATENT
2-Chloro-4-aminotoluene-S-sulfonic acid
LOCATION: Japan.
ASSIGNEE: Dainippon Ink and Chemicals, Inc.; Kawamura
Physical and Chemical Research Institute
PATENT: Japan Kokai Tokkyo Koho ; JP 8221659 A2 ; JP
8821659 DATE: 830203
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DIALOG File J99: CA SEARCH 1987-1988 UD=10810 (Coltr. 1088 by the Amer. Chem. Soc.)

APPLICATION: JP 81119093 (810731)
PAGES: 2 PP. CODEN: JXKXAF LANGUAGE: Japanese CLASS: C07C
143/63; C07C-139/00

98083403 CA: 98(7)83403p PATENT

Aminoarylsulfonic acids
INVENTOR(AUTHOR): Emde, Herbert; Blank, Heinz Ulrich; Schnogg
G. Peter
LOCATION: Fed. Rep. Ger.
ASSIGNEE: Bayer A.-G.
PATENT: Germany Offen.; DE 3114830 A1 DATE: 821104
APPLICATION: DE 3114830 (810411)
PAGES: 34 PP. CODEN: GXKXBX LANGUAGE: German CLASS: C07C-
139/00; C07C-143/58; C07C-143/60; C07C-143/63; C07C-143/64

98083402 CA: 98(7)83402n PATENT

Aromatic aminoarylsulfonic acids
INVENTOR(AUTHOR): Emde, Herbert; Blank, Ulrich; Schnogg,
Peter
LOCATION: Fed. Rep. Ger.
ASSIGNEE: Bayer A.-G.
PATENT: Germany Offen.; DE 3114829 A1 DATE: 821104
APPLICATION: DE 3114829 (810411)
PAGES: 27 PP. CODEN: GXKXBX LANGUAGE: German CLASS: C07C-
139/00; C07C-143/58; C07C-143/63

98021815 CA: 98(4)21815b PATENT

Dyeing the fleeces of skins
INVENTOR(AUTHOR): Murgesser, Thilo; Lemm, Gunther
LOCATION: Fed. Rep. Ger.
ASSIGNEE: BASF A.-G.
PATENT: European Pat. Appl.; EP 35172 A2 DATE: 810809
APPLICATION: EP 8110161 (810219) *DE 3007628 (800229)
PAGES: 33 PP. CODEN: EPXXDW LANGUAGE: German CLASS: D06P-
003/30; D06P-001/02; C09B-029/42; C09B-029/52
DESIGNATED COUNTRIES: CH; DE; FR; GB; IT

98008123 CA: 98(2)8123v PATENT

Monazo or disazo pigments
INVENTOR(AUTHOR): Pechey, David Thomas; Coy, John Hugh
LOCATION: Swiss
ASSIGNEE: Ciba-Geigy A.-G.
PATENT: European Pat. Appl.; EP 29009 A1 DATE: 810523
APPLICATION: EP 80810339 (801103) *GB 79/38591 (791107)
PAGES: 29 PP. CODEN: EPXXDW LANGUAGE: German CLASS: C09B-
041/00; C09B-067/22; C09B-029/00; C09B-035/00; C09B-035/10
DESIGNATED COUNTRIES: CH; DE; FR; GB; IT

94113975 CA: 94(14)113975f JOURNAL

Thin-layer chromatography of aromatic amines on ammonium
selydiphosphate and tungstophosphate
AUTHOR(S): Lepri, L.; Desideri, P. G.; Heimler, D.

LOCATION: Inst. Anal. Chem., Univ. Florence, Florence, Italy
JOURNAL: J. Chromatogr. DATE: 1981 VOLUME: 207 NUMBER: 1
PAGES: 29-36 CODEN: JOCRAH ISSN: 0021-9673 LANGUAGE: Engl
1st

92221921 CA: 92(24)221921y PATENT

Modifier composition for azo pigments based on
2-hydroxy-3-naphthoic acid
INVENTOR(AUTHOR): Burley, David R.; Brookes, Christopher J.
LOCATION: USA
ASSIGNEE: American Cyanamid Co.
PATENT: United States US 4224221 DATE: 800923
APPLICATION: United States US 1988 DATE: 790108
PAGES: 6 PP. CODEN: USKXAM LANGUAGE: English CLASS: 2000-
2000; C09B-029/10;

93208183 CA: 93(22)208183f PATENT

Azo pigments
INVENTOR(AUTHOR): Von, Isaiah
LOCATION: USA
ASSIGNEE: American Cyanamid Co
PATENT: United States US 4217273 DATE: J00812
APPLICATION: United States US 1567 DATE: 790108
PAGES: 4 PP. CODEN: USKXAM LANGUAGE: English CLASS: 2000-
2000; C09B-029/04;

93098780 CA: 93(10)98780y PATENT

Dialysis monitored preparation of azal salts of azo
pigments
INVENTOR(AUTHOR): Hamilton, Alexander; Nelson, Colin
LOCATION: USA
ASSIGNEE: Ciba-Geigy Corp.
PATENT: United States US 4190878 DATE: 800228
APPLICATION: Britain GB 7644771 DATE: 761028
PAGES: 3 PP. CODEN: USKXAM LANGUAGE: English CLASS: 260 3
0000; C09B-045/14; C09B-045/16; C09B-045/18; C09B-045/20;

93073767 CA: 93(8)73767k PATENT

Dyeing of thermoplastic materials
INVENTOR(AUTHOR): Guenther, Paul; Henning, Georg
LOCATION: Fed. Rep. Ger.
ASSIGNEE: BASF A.-G.
PATENT: Germany Offen. DE 2845073 DATE: 800809
APPLICATION: Germany DE 2845073 DATE: 781017
PAGES: 12 PP. CODEN: GXKXBX LANGUAGE: German CLASS: C08K-
003/20; C08K-005/00;

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DIALOG File 200: CA SEARCH 1007-1008 US-10010 (Copr. 1966 by the Amer. Chem. Soc.)

9007791 CA: 90(8)75761d PATENT
Calcium lake with high brilliance
INVENTOR(AUTHOR): Hennrich, Georg; Guenther, Paul
LOCATION: Fed. Rep. Ger.
ASSIGNEE: BASF A.-G.
PATENT: France Demande FR 2422208 DATE: 80-229
APPLICATION: Germany DE 2824028 DATE: 780803
PAGES: 21 pp. CODEN: PRXHX LANGUAG: French CLASS: C09B-029/28; C09B-043/00; C09B-082/00; C09B-017/00;

9219205 CA: 92(22)182028n PATENT
Calcium lake with good covering power, high pure tone
brilliance, and improved fastness to light
INVENTOR(AUTHOR): Hennrich, Georg; Guenther, Paul
LOCATION: Fed. Rep. Ger.
ASSIGNEE: BASF A.-G.
PATENT: Germany Offen. DE 2824028 DATE: 802221
APPLICATION: Germany DE 2824028 DATE: 780803
PAGES: 26 pp. CODEN: GRXXHX LANGUAG: German CLASS: C09B-043/00; C09B-029/10; C09B-082/00; C09B-017/00;

9008091 CA: 90(8)08091j PATENT
Rubine mixed strontium-cadmium salts of
3-chloro-6-methyl-8-sulphanylacetylenephthalic acid
INVENTOR(AUTHOR): Putney, Richard Knight
LOCATION: USA
ASSIGNEE: Hercules Inc.
PATENT: United States US 419977 DATE: 780919
APPLICATION: United States US 764222 DATE: 770131
PAGES: 7 pp. CODEN: USXXAM CLASS: 260151020; C09D-048/12;

90024780 CA: 90(4)24780p PATENT
Azo dye
LOCATION: USA
ASSIGNEE: Hercules Inc.
PATENT: Netherlands Appl. NL 7712762 DATE: 780602
APPLICATION: United States US 764222 DATE: 770131
PAGES: 14 pp. CODEN: MAXXAM CLASS: C09B-019/22;

96148144 CA: 96(18)148144h PATENT
Azo pigments
INVENTOR(AUTHOR): Stefanosik, Ernest Anton
LOCATION: USA
ASSIGNEE: du Pont de Nemours, E. I., and Co.
PATENT: Germany Offen. DE 2802990 DATE: 781802
APPLICATION: United States US 764224 DATE: 770131
PAGES: 10 pp. CODEN: GRXXHX CLASS: C09B-019/14;

87117877 CA: 87(18)117877p PATENT
2-chloro-6-toluidino-6-sulfonic acid
INVENTOR(AUTHOR): Noguchi, Kazuo; Fukuda, Tetsuo; Mitatsuyan
agi, Shunichi

LOCATION: Japan
ASSIGNEE: Dainichiseika Color and Chemicals Mfg. Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho JP 7762240 DATE: 770923
APPLICATION: Japan JP 75125714 DATE: 751112
PAGES: 4 pp. CODEN: JXXKAF CLASS: C07C-142/56;

90002395 CA: 90(2)002395 PATENT
Azo dyes converted into a lake
INVENTOR(AUTHOR): Landler, Josef; Moerfel, Erhard
LOCATION: Ger.
ASSIGNEE: Hoechst A.-G.
PATENT: Germany DE 2502791 DATE: 760729
APPLICATION: Germany DE 2502791 DATE: 750130
PAGES: 9 pp. CODEN: GRXXAM CLASS: C09B-029/00;

90072082 CA: 90(14)72082v PATENT
Red azo pigment lakes
INVENTOR(AUTHOR): Dobrovolny, Jan; Kreidl, Zdenek; Najek,
Ladislav
PATENT: Czechoslovakia CS 147169 DATE: 720115
APPLICATION: Czechoslovakia CS 312470 DATE: 700606
PAGES: 4 pp. CODEN: CZXXA9 CLASS: C 09B

79060220 CA: 79(14)90220y PATENT
Azo pigment toners
INVENTOR(AUTHOR): Price, David; Hamilton, Alexander; Bradley
Gordon Frank
ASSIGNEE: Ciba-Geigy (UK) Ltd.
PATENT: Britain GB 1312147 DATE: 730411
APPLICATION: Britain GB 383170 DATE: 700606
PAGES: 6 pp. CODEN: BRXXAA CLASS: C 09B

78127822 CA: 78(22)127822i PATENT
Nucleo lacquers
INVENTOR(AUTHOR): Dobrovolny, Jan
PATENT: Czechoslovakia CS 145975 DATE: 721015
APPLICATION: Czechoslovakia CS 623169 DATE: 690918
PAGES: 4 pp. CODEN: CZXXA9 CLASS: C 09B

98077908 CA: 98(17)77949g PATENT
3-Chloro-4-toluidino
INVENTOR(AUTHOR): Doering, Arthur A.
ASSIGNEE: American Cyanamid Co.
PATENT: United States US 3341595 DATE: 870912
APPLICATION: United States DATE: 841001
PAGES: 4 pp. CODEN: USXXAM CLASS: 260-590



Hill Top Research, Inc.

P.O. Box 42501, Cincinnati, Ohio 45242 (513) 831-3114

Ref.: 81-0962-21

August 24, 1981

ACUTE ORAL TOXICITY,
PRIMARY SKIN IRRITATION AND ACUTE EYE
IRRITATION POTENTIALS OF IC-2

For BASF Wyandotte Corporation

PURPOSE

This study was conducted to evaluate the acute oral toxicity and the primary skin and eye irritation potentials of the test material in accordance with the techniques specified in the Federal Insecticide, Fungicide, and Rodenticide Act (40 CFR 163).

TEST MATERIAL

The sample was received from BASF Wyandotte Corporation on July 13, 1981 for use in these studies. IC-2, Lot #91749243 is a gray powder with a pH of 2.6 when moistened with water, which was stored in a closed metal can at room temperature throughout the study period.

PROCEDURE

Acute Oral Administration - Rats

The test sample was administered on August 3, 1981 orally by esophageal intubation to one group composed of five male and five female Sprague-Dawley derived albino rats from Hilltop Lab Animals, Inc. Weight range for the male rats was 212 to 240 grams and for the female rats was 202 to 215 grams. The sample was administered as a 50.0% w/v solution in distilled water, at a dosage level of 5.0 grams per kilogram of body weight.



Food was withheld from the rats for 19 1/4 to 19 1/2 hours prior to dosing. Following dosing, food consisting of Purina Laboratory Chow and water were available ad libitum. The rats were housed in groups in stainless steel wire mesh cages suspended above the droppings. The animals were housed under a 12-hour light/12-hour dark cycle. The rats were acclimated to the laboratory for six days prior to dosing and were individually identified by numbered ear tags and tail marks with permanent ink. All animals were observed closely for gross signs of systemic toxicity and mortality at one-half to three-quarter, one and one-half to two, and four and one-quarter to four and one-half hours during the day of dosing, and once or twice daily thereafter for a total of 14 days. The protocol specified observations twice daily. The rats were not weighed on the seventh post-dosing day in deviation from the protocol. At the end of the 14-day observation period the rats were weighed, sacrificed by CO₂ inhalation and gross necropsies were performed.

Patch Test for Primary Skin Irritation and Corrosivity - Rabbits

Five-tenths gram of the test material moistened with physiologic saline was applied on July 22, 1981, under a one-inch square surgical gauze patch, two layers thick, to two intact skin areas and two abraded skin areas on each of the six New Zealand White rabbits from Johnson Bunny Ranch. The rabbits were acclimated to the laboratory for eight days prior to dosing and were individually identified by means of numbered ear tags. The application sites were prepared by clipping the hair from the saddle area of the rabbits. The abraded areas were prepared by making minor epidermal incisions with a hypodermic needle. The abrasions were sufficiently deep to penetrate the epidermis, but not to induce bleeding. Each patch was held in place with two strips of one-inch adhesive tape. After application of the patches, the trunk of each rabbit was wrapped with rubber dental damming, which was secured with staples. An outer layer of gauze and tape was placed around the trunk of each animal. The animals were restrained in Newmann harnesses for 24 3/4 hours.

At the end of the exposure period, the patches were removed and any residual sample was gently sponged from the skin with a moistened towel. The reactions were scored immediately after removal of the patches (24-hour reading), and again two days later at approximately 70 hours post-dosing (72-hour reading), according to the scale reproduced in Table 1 accompanying this report. Animals were not held after the 72-hour reading to allow examination of animals with potentially reversible irritation in deviation from the protocol.

Acute Eye Application - Rabbits

One-tenth gram of the undiluted sample was applied on July 23, 1981, to the left or right eye of each of nine New Zealand White rabbits from Hilltop Lab Animals, Inc. and J & J Research Farms, Inc. The opposite eyes were untreated and served as controls. The treated eyes of six rabbits were left unrinsed. The treated eyes of three rabbits were rinsed after 30 seconds, for 60 seconds with at least 200 ml of lukewarm tap water. Examinations for gross signs of eye irritation were made at approximately 22 3/4 to 23, 44, and 69 to 69 1/4 hours and 4, 7, 10, 13, 16, 19, and 21 days following application. Fluorescein Sodium Ophthalmic Solution (2.0%) was used to assess the eyes approximately 24-hours before the study and to aid in scoring of the rabbits which had scores of 3 or less in corneal opacity during the study. The protocol required fluorescein applications only for scores of 2 or below. Fluorescein was inadvertently not used to score Rabbit Nos. 5-9 on day 16. Scoring of irritative effects was according to the method of Draize, in which corneal, iris, and conjunctival effects are scored separately¹. This method is reproduced in the addendum following Table 3.

One rabbit, No. 1, was found dead on day 4, was necropsied but was not replaced.

RESULTSAcute Oral Administration - Rats

No mortalities occurred at the dosage level tested. Therefore, the acute oral LD₅₀ value was found to be greater than 5.0 g/kg of body weight for male and female Sprague-Dawley derived albino rats.

Both male and female groups exhibited normal behavior and appearance during the day of dosing and throughout the observation period.

Gross necropsies performed upon the termination of the study revealed no gross pathological alterations or lesions in any rats tested.

All male and female rats gained weight during the observation period. Body weight data are given below:

Dosage g/kg	Ear Tag No.	Sex	Body Weight		
			Start g	Finish g	Gain g
5.0	4538	M	240	339	99
5.0	4539	M	231	333	102
5.0	4540	M	212	341	129
5.0	4541	M	225	362	137
5.0	4542	M	224	320	106
		Mean (S.D.)	226 (10)	341 (13)	115 (17)

¹ Draize, J. H., 1959. "Dermal Toxicity", in Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Staff of the Division of Pharmacology of the Federal Food and Drug Administration (Austin, Texas: The Editorial Committee of the Association of Food and Drug Officials of the United States), p. 51.

<u>Dosage</u> g/kg	<u>Ear Tag No.</u>	<u>Sex</u>	<u>Body Weight</u>		
			<u>Start</u> g	<u>Finish</u> g	<u>Gain</u> g
5.0	4573	F	204	236	32
5.0	4574	F	215	259	44
5.0	4575	F	211	248	37
5.0	4576	F	202	249	47
5.0	4577	F	213	251	38
		Mean (S.D.)	209 (6)	249 (8)	40 (6)

Primary Skin Irritation Test - Rabbits

The results of a 24-hour patch of IC-2 to the intact and abraded skin areas on the backs of New Zealand white rabbits are presented in Table 1.

Irritative effects noted during the course of the study included very slight erythema, observed at abraded and intact sites of three animals. Slight erythema was noted on abrasions in five animals. Very slight edema was found in one animal. No evidence of corrosivity was observed.

The Primary Irritation Index was found to be 0.3.

Acute Eye Irritation - Rabbits

The results following application of IC-2 to the non-rinsed and rinsed eyes of New Zealand White rabbits are presented in Tables 2 and 3, respectively. No gross pathology was observed upon necropsy of rabbit (No. 1) from the non-rinsed eye group which died by the fourth day after dosing. The death was not clearly attributable to sample administration although irritation in this animal was more serious than in any other rabbit.

Non-Rinsed Eyes

Irritative effects noted during the study included corneal opacity, iritis, and irritation. Mild to severe corneal opacity was noted with from one-quarter or less up to the entire corneal area involved in all rabbits tested. Corneal opacity persisted through the 72-hour reading in all rabbits and occurred sporadically thereafter in individual animals. Mild to severe iritis was observed in all animals, persisting mainly through the 48-hour reading. Conjunctival effects included mild to severe erythema, edema, and discharge, as well as extreme edema in one animal. Conjunctival effects lasted through day 4 of the observation period in most rabbits. The total irritation scores of this group ranged from 0 to 90.

Rinsed Eyes

Sporadic occurrences of mild corneal opacity were observed in the rinsed eye group with the aid of fluorescein staining in two rabbits. One rabbit had sporadic occurrences of minor iritis. Conjunctival effects included very slight to severe erythema and edema. No significant change persisted more than seven days. Total irritation scores ranged from 0-12 in this group throughout the observation period.

Ref.: 81-0962-21

August 24, 1981

SUMMARY

The acute oral toxicity, primary skin irritation and corrosivity, and acute eye irritation potentials of IC-2 were evaluated in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (40 CFR 165).

The acute oral LD₅₀ value was found to be greater than 5.0 g/kg. No systemic signs of toxicity were noted in any animals throughout the study.

Following a 24-hour patch application of IC-2 to the intact and abraded skin areas on the back of New Zealand White rabbits, the Primary Irritation Index was found to be 0.3. Slight erythema and edema were observed in some of the animals. No evidence of corrosivity was observed.

Application of IC-2 to the unrinsed eyes of New Zealand White rabbits produced corneal opacity, iritis changes, and conjunctival effects (erythema, edema, and discharge). The changes were mostly reversible within seven days after application. One rabbit died before the four-day reading. This rabbit exhibited severe effects at the 24-, 48-, and 72-hour readings. The death was not clearly application-related. In rinsed eyes, effects were mainly limited to conjunctival effects and were minimal by the 4-day reading in all rabbits.

Based on these results, IC-2 is classified in Category IV as non-toxic by oral ingestion, in Category IV for skin effects, and probably in Category II for eye effects as these terms are defined in the above-cited Regulations.

Approved by:

Submitted by:

David L. Conine, Ph.D.
Study Director, Toxicology

Alan S. Weiner, B.A.
Technical Writer, Toxicology

Reviewed by Quality Assurance:

Gayle K. Mulberry, R. S. Agri
Director, Quality Assurance

Table 1

Primary irritation scores in New Zealand White rabbits following a 24-hour patch application of IC-2.

Skin	Time Hours	Score for Rabbit Number*												Total Score	Average	
		1	2	3	4	5	6	1	2	3	4	5	6			
<u>Erythema and Eschar Formation</u>																
		<u>Site</u>	<u>A</u>	<u>C</u>	<u>B</u>	<u>D</u>	<u>C</u>	<u>A</u>	<u>D</u>	<u>B</u>	<u>A</u>	<u>C</u>	<u>B</u>	<u>D</u>		
Intact	24		0	0	0	1	0	0	1	0	1	1	0	0	4	0.33
	72		0	0	0	1	0	0	1	0	1	0	0	0	3	0.25
		<u>Site</u>	<u>B</u>	<u>D</u>	<u>C</u>	<u>A</u>	<u>D</u>	<u>B</u>	<u>A</u>	<u>C</u>	<u>B</u>	<u>D</u>	<u>C</u>	<u>A</u>		
Abraded	24		0 ^a	0 ^a	0	1	0 ^a	0 ^a	0 ^a	0 ^a	1 ^a	1 ^a	0 ^a	0 ^a	3	0.25
	72		0 ^a	0 ^a	0	1	0 ^a	0 ^a	0 ^a	0	1 ^a	0 ^a	0 ^a	0 ^a	2	0.17
<u>Edema Formation</u>																
		<u>Site</u>	<u>A</u>	<u>C</u>	<u>B</u>	<u>D</u>	<u>C</u>	<u>A</u>	<u>D</u>	<u>B</u>	<u>A</u>	<u>C</u>	<u>B</u>	<u>D</u>		
Intact	24		0	0	0	0	0	0	0	0	1	0	0	0	1	0.08
	72		0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
		<u>Site</u>	<u>B</u>	<u>D</u>	<u>C</u>	<u>A</u>	<u>D</u>	<u>B</u>	<u>A</u>	<u>C</u>	<u>B</u>	<u>D</u>	<u>C</u>	<u>A</u>		
Abraded	24		0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
	72		0	0	0	0	0	0	0	0	0	0	0	0	0	0.00

Primary Irritation Index 0.3

^aAbrasions slightly red.

SCORING KEY

Evaluation of Skin Reactions	Value*
<u>Erythema and eschar formation:</u>	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
<u>Edema formation:</u>	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)	4

August 24, 1981

Table 2

Eye irritation scores in albino rabbits following application of 0.1 ml of IC-2 without a subsequent rinse.

Rabbit Number	Time Hours	Cornea		Iris	Conjunctivae			Total Score*
		Opacity	Area		Erythema	Swelling	Discharge	
1	24 hours	3/3 ^a	3/3	2	3	2	3	71
	48 hours	3/3	4/3	2	3	3	3	88/73
	72 hours	3/NA ^b	4/NA	2	3	4	3	90
	4 days	ND ^c	ND	ND	ND	ND	ND	ND
2	24 hours	1/1	2/2	1	3	2	3	31
	48 hours	1/1	2/1	1	3	3	3	33/2 ^a
	72 hours	1/0	2/0	0	3	1	2	22/12
	4 days	0/0	0/0	0	2	0	0	4
	7 days	0/0	0/0	0	1	0	0	2
	10 days	0/1	0/1	0	1	0	0	2/7
	13 days	0/0	0/0	0	0	0	0	0
	16 days	0/0	0/0	0	0	0	0	0
	19 days	0/0	0/0	0	0	0	0	0
	21 days	0/0	0/0	0	0	0	0	0
3	24 hours	1/1	3/2	2	3	3	3	43/38
	48 hours	1/1	2/1	1	3	2	1	27/22
	72 hours	0/1	0/2	0	2	1	1	8/18
	4 days	0/0	0/0	0	2	1	0	6
	7 days	0/0	0/0	0	1	0	0	2
	10 days	0/0	0/0	0	2	1	0	6
	13 days	0/0	0/0	0	1	0	0	2
	16 days	0/0	0/0	0	1	0	0	2
	19 days	0/0	0/0	0	1	0	0	2
	21 days	0/0	0/0	0	0	0	0	0

^aScores without/with fluorescein application.

^bNA - Sodium Fluorescein not used in scoring due to severity of corneal opacity by visual examination.

^cND = No data, rabbit found dead on day 4.

*Total score is the sum of the following three sub-totals:

(a) degree of opacity x area involved x 5

(b) iris score x 5

(c) sum of scores for erythema, swelling, and discharge x 2

Total possible score = 110

Table 2 (Cont.)

Eye irritation scores in albino rabbits following application of 0.1 ml of IC-2 without a subsequent rinse.

Rabbit Number	Time Hours	Cornea		Iris	Conjunctivae			Total Score ^a
		Opacity	Area		Erythema	Swelling	Discharge	
4	24 hours	1/1 ^a	2/2	2	3	2	3	36
	48 hours	1/1	2/2	1	3	2	2	29
	72 hours	1/1	2/1	0	2	0	1	16/11
	4 days	3/3	1/1	1	2	0	0	24
	7 days	1/0	1/0	0	1	0	0	7/2
	10 days	2/0	1/0	0	0	0	0	10/0
	13 days	0/0	0/0	0	0	0	0	0
	16 days	0/0	0/0	0	0	0	0	0
	19 days	0/1	0/1	0	0	0	0	0/5
	21 days	0/1	0/1	0	0	0	0	0/5
5	24 hours	0/1	0/1	2	3	2	1	22/27
	48 hours	1/1	1/1	2	2	1	1	23
	72 hours	1/0	1/0	1	2	0	0	14/9
	4 days	0/0	0/0	1	1	0	0	7
	7 days	0/0	0/0	0	0	0	0	0
	10 days	0/0	0/0	0	1	0	0	2
	13 days	0/0	0/0	0	0	0	0	0
	16 days	0/0	0/0	0	0	0	0	0
	19 days	0/0	0/0	0	0	0	0	0
	21 days	0/0	0/0	0	0	0	0	0
6	24 hours	1/1	1/1	2	3	3	1	29
	48 hours	1/1	1/1	1	2	1	1	18
	72 hours	1/0	2/0	0	2	1	1	18/8
	4 days	0/0	0/0	0	1	1	0	4
	7 days	0/0	0/0	0	1	0	0	2
	10 days	0/1	0/1	0	0	0	0	0/5
	13 days	0/0	0/0	0	0	0	0	0
	16 days	0/ ^b	0/ ^b	0	0	0	1	2
	19 days	0/1	0/1	0	0	0	0	0/5
	21 days	0/1	0/1	0	0	0	0	0/5

^aScores without/with fluorescein application.

^bRabbit eyes were not scored with Sodium Fluorescein Ophthalmic Solution on day 16 due to lack of dye.

*Total score is the sum of the following three sub-totals:

(a) degree of opacity x area involved x 5

(b) iris score x 5

(c) sum of scores for erythema, swelling, and discharge x 2

Total possible score = 110

Table 3

Eye irritation scores in albino rabbits following application of 0.1 ml of IC-2 with a subsequent lukewarm tapwater rinse (200 ml for 60 seconds after 30 seconds of sample contact).

Rabbit Number	Time Hours	Cornea		Iris	Conjunctivae			Total Score ^a
		Opacity	Area		Erythema	Swelling	Discharge	
7	24 hours	0/0 ^a	0/0	0	3	2	1	12
	48 hours	0/0	0/0	0	2	1	0	8
	72 hours	0/0	0/0	0	2	0	0	4
	4 days	0/0	0/0	0	1	1	0	4
	7 days	0/0	0/0	0	1	0	0	2
	10 days	0/0	0/0	0	1	0	0	2
	13 days	0/0	0/0	0	1	0	0	2
	16 days	0/ b	0/ b	0	0	0	0	0
	19 days	0/0	0/0	0	0	0	0	0
21 days	0/0	0/0	0	0	0	0	0	
8	24 hours	0/0	0/0	0	3	0	0	6
	48 hours	0/0	0/0	0	1	0	0	2
	72 hours	0/0	0/0	0	1	0	0	2
	4 days	0/0	0/0	0	1	1	0	4
	7 days	0/0	0/0	0	0	0	0	0
	10 days	0/1	0/1	0	0	0	0	0/5
	13 days	0/0 ^b	0/0 ^b	0	1	0	0	2
	16 days	0/ b	0/ b	0	0	0	0	0
	19 days	0/0	0/0	0	0	0	0	0
21 days	0/1	0/1	0	0	0	0	0	
9	24 hours	0/0	0/0	1	2	0	0	9
	48 hours	0/0	0/0	1	2	0	0	9
	72 hours	0/0	0/0	1	2	0	0	9
	4 days	0/0	0/0	1	1	0	0	7
	7 days	0/0	0/0	1	1	0	0	7
	10 days	0/1	0/1	0	0	0	0	0/5
	13 days	0/0 ^b	0/0 ^b	1	0	0	0	5
	16 days	0/ b	0/ b	0	0	0	0	0
	19 days	0/0	0/0	0	0	0	0	0
21 days	0/0	0/0	1	0	0	0	5	

^aScore = without/with fluorescein application

^bRabbit eyes were not scored with Sodium Fluorescein Ophthalmic Solution on day 16 due to lack of dye.

^aTotal score is the sum of the following three sub-totals:

(a) degree of opacity x area involved x 5

(b) iris score x 5

(c) sum of scores for erythema, swelling, and discharge x 2

Total possible score = 110

ADDENDUM

Scale for Scoring Ocular Lesions

- (1) Cornea
 - (a) Opacity-degree of density (area most dense taken for reading)
 - No Capacity.....0
 - Scattered or diffuse area, details of iris clearly visible (mild cornea opacity)1
 - Easily discernible translucent areas, details of iris slightly obscured (moderate)2
 - Opalescent areas, no details of iris visible, size of pupil barely discernible (severe).....3
 - Opaque, iris invisible (extreme).....4
 - (b) Area of cornea involved
 - One quarter (or less) but not zero.....1
 - Greater than one quarter, but less than half.....2
 - Greater than half, but less than three quarters.....3
 - Greater than three quarters, up to whole area.....4

A X B X 5 Total Maximum = 80
- (2) Iris
 - (a) Values
 - Normal.....0
 - Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive) (mild iritis).....1
 - No reaction to light, hemorrhage, gross destruction (any or all of these) (severe iritis).....2

A X 5 Total Maximum = 10
- (3) Conjunctivae
 - (a) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)
 - Vessels normal.....0
 - Vessels definitely injected above normal (slight, mild).....1
 - More diffuse, deeper crimson red, individual vessels not easily discernible (moderate).....2
 - Diffuse beefy red (severe).....3
 - (b) Chemosis
 - No swelling.....0
 - Any swelling above normal (includes nictitating membrane) (slight).....1
 - Obvious swelling with partial eversion of lids (moderate).....2
 - Swelling with lids about half closed (severe).....3
 - Swelling with lids about half closed to completely closed (extreme).....4
 - (c) Discharge
 - No discharge.....0
 - Any amount different from normal (does not include small amounts observed in inner canthus of normal animals (mild or slight).....1
 - Discharge with moistening of the lids and hairs just adjacent to lids (moderate).....2
 - Discharge with moistening of the lids and hairs, and considerable area around the eye (severe).....3

Score (A + B + C) X 2 Total Maximum = 20



Hill Top Research, Inc.

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IMPORTANT NOTICE

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STUDY REVIEW RECORD

Reference: 81-0962-21

Date of Study Initiation: July 22, 1981

Date of Study Completion: August 17, 1981

Study Director: David L. Conine, Ph.D.

Project Leader: Catherine M. Bans, B.A.

Location of Specimen Storage: N/A

Disposition of Remaining Test Material: At the conclusion of a test program, any unused portion of each sample used will be stored at Hill Top Research, Inc. No materials will be maintained longer than six months after the completion of the study unless the client notifies Hill Top Research, Inc. New drugs are exempt from the above procedure. They will be retained or returned to the client.

Location of Raw Data: Hill Top Research, Inc.
P. O. Box 138
Miamiville, Ohio 45147

Location of Final Report: Hill Top Research, Inc.

Quality Assurance Unit Statement

Date(s) Study Inspected: August 3, 1981

Date Report Reviewed: September 16, 1981

Date(s) Findings Reported to Management: August 3, 1981

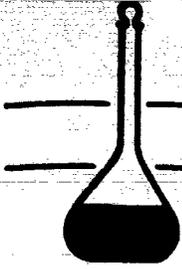
Date(s) Findings Reported to Study Director: August 3, 1981

Wally Ammann
Quality Assurance Auditor

September 16, 1981
Date

Robert L. Dyle
Quality Assurance Director

9/18/81
Date



Hill Top Research, Inc.

P.O. Box 42591, Cincinnati, Ohio 45242 (513) 831-3114

The Salmonella/Microsomal Assay for Bacterial
Mutagenic Activity of IC-2 Lot #91749243

For: BASF Wyandotte Corporation

Reference: 81-0960-21



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I. SAMPLE DATA AND CHARACTERISTICS

- A. DATE SAMPLE RECEIVED: 7/13/81
- B. HILL TOP RESEARCH PROJECT NUMBER: 81-0960-21
- C. SAMPLE IDENTIFICATION: IC-2 Lot #91749243
- D. SAMPLE CHARACTERISTICS:
 - 1. Physical Description: Sample IC-2 Lot #91749243 is a solid
 - 2. Molecular Weight: Not applicable
 - 3. Solvent System: DMSO
 - 4. Solubility: Not applicable
- E. SAMPLE STORAGE CONDITIONS: Room temperature protected from direct light.
- F. DATE STUDY STARTED: 7/16/81
- G. DATE STUDY FINISHED: 7/24/81

II. STUDY AUTHORIZATION AND APPROVAL

- A. HILL TOP RESEARCH PROJECT NUMBER: 81-0960-21
- B. DATE REPORTED: 7/28/81
- C. PRINCIPAL INVESTIGATOR REPRESENTING CLIENT:
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Industrial Toxicologist
- D. STUDY DIRECTOR AT HILL TOP RESEARCH:
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Study Director, Genetic Toxicology
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Mark R. Entrup 8/13/81
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- F. STUDY APPROVED BY:

George C. Lavelle 9/1/81
George C. Lavelle, Ph.D.
Director of Technical Services,
Genetic Toxicology

III. SUMMARY

Test article IC-2 Lot #91749243 was tested in the Salmonella/Microsomal Mutagenicity Plate Incorporation Assay with Salmonella tester strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 at doses of 1 ug, 10 ug, 100 ug, 500 ug, and 1000 ug per plate both without and with the S-9 metabolic activation system.

Under conditions of this investigation, no mutagenic activity was demonstrated for test article IC-2 Lot #91749243.

IV. INTRODUCTION

The Salmonella/Microsomal Assay for Mutagenesis, developed for the detection of potential chemical mutagens and carcinogens by Bruce N. Ames, Ph.D. of the University of California at Berkeley, is probably the most widely adopted, rapid, in vitro, microbial screening system yet developed for this purpose. Based upon the premise that DNA of microorganisms and mammals is essentially the same structurally and chemically and the theory that most carcinogens cause cancer through somatic mutation, in vitro microbial mutagenesis assays such as the "Ames" test serve as a valuable screening tool in the assessment of the mutagenic potential of a chemical for humans. While they can be used to identify chemicals with biological properties similar to known carcinogens and to predict with high probability that a chemical will produce cancer in mammals, in vitro mutagenesis assays are only presumptive and cannot be considered as definitive tests in the identification of carcinogens.

Since bacteria have such relatively short generation times and are sensitive to a wide range of mutagenic substances, they are well suited for use in the initial screening for mutagenic agents in large numbers of compounds. The majority of the tests, including the "Ames" test are designed to detect reverse mutations such as reversion of selected auxotrophic bacteria (strains which require a particular nutrient such as an amino acid for growth) to prototrophic or wild-type strains. Since reverse mutations of this type indicate a change at only one gene or nucleotide sequence in the DNA, this is referred to as a point mutation. Such mutations are the results of either base-pair substitutions or frameshift mutations (addition or deletion of a single nucleotide pair).

The Salmonella/Microsomal Mutagenicity Assay, in particular, utilizes specially constructed mutants of Salmonella typhimurium which have been selected for their sensitivity and specificity in being reverted from histidine dependence back to the wild-type or histidine independence. Consequently, by demonstrating a significant increase in the number of revertant mutant colonies occurring in cultures treated with the test compound as compared to the spontaneous mutants (background count) obtained in untreated control cultures, mutagenic activity of the test compound is determined. Detection of promutagens (compounds which require metabolic conversion by mammalian enzyme systems to their reactive forms) is accomplished in this assay by adding liver microsomal enzyme preparations (S-9) to the test system, thus, incorporating an important aspect of mammalian metabolism into an in vitro test.

V. PURPOSE

The purpose of this study was to evaluate the mutagenic potential of the test material using the Salmonella/Microsomal Mutagenesis Assay.

VI. MATERIALS

A. Bacterial Indicator Strains:

Salmonella typhimurium, strains TA 1535
TA 1537
TA 1538
TA 98
TA 100

B. Animals:

Sprague-Dawley Rats
Sex: adult males
Weight upon receipt: 175-190 g.
Weight upon sacrifice: 200-275 g.

C. Media and Reagents:

1. Nutrient broth (Difco) supplemented with 0.5% NaCl
2. Top agar (0.6% Difco agar, 0.5% NaCl, 0.5 mM biotin, and 0.5 mM L-histidine-HCl)
3. Minimal-glucose agar medium (1.5% Bacto-Difco agar in Vogel-Bonner Medium E, 2% glucose)
4. Tryptone agar medium
5. Arcclor 1254
6. DMSO
7. Glucose-6-phosphate
8. Nicotinamide adenine dinucleotide phosphate (NADP)

D. Positive Mutagenic Controls:

1. Nonactivation assay: N-methyl-N-nitro-N-nitrosoguanidine (MNNG)
9-Aminoacridine (9-AA)
2-Nitrofluorene (2-NF)
2. Activation assay: 2-Aminoanthracene (2-AA)

VII. METHODS

A. Bacterial Preparation:

Upon receipt of the indicator strains (obtained from Bruce N. Ames), nutrient broth (containing 0.5% NaCl) cultures were prepared. All strains were checked for the appropriate mutation markers. The cultures were then frozen, 0.8 ml nutrient broth culture with 0.07 ml DMSO, and stored at -80°C . Each week, new cultures were started from the frozen permanents in 10 ml of nutrient broth with 0.5% NaCl and incubated on a shaker overnight at 37°C . These cultures were then stored in a refrigerator and checks for the appropriate genetic markers were conducted before each culture was released for use in testing. Such cultures were only used for one week.

B. Preliminary Toxicity Testing:

The effect of the test articles on the survival of the bacterial strains was determined prior to the Ames Bioassay. This was accomplished by adding different levels of the test articles and solvent to tubes containing 2.0 ml top agar (at 45°C) and 0.1 ml of the tester strain. After mixing, the tube contents were poured onto the surface of tryptone agar plates. The plates were then incubated at 37°C for 18-24 hours and the background lawn of bacteria in test articles plates was compared to the bacterial lawn in the solvent plates. Toxicity on the tryptone agar plates was detectable by a thinning or disappearance of this background lawn of bacteria.

C. Plate Assay for the Detection of Direct Acting Mutagens:

All plate assays for bacterial mutagenesis were conducted according to the methodology described by B. N. Ames, et. al. (1)

The following were added, in order, to 2 ml of molten top agar contained in 13 x 100 mm tubes:

1. 0.1 ml of a log phase culture of the appropriate indicator strain
2. 0.1 ml of the appropriate concentration of test material

VII. METHODS (Continued)

Each tube was prepared individually, immediately vortexed, and the contents poured over the surface of a minimal-glucose agar plate. Each plate was rotated to evenly distribute the top agar before it hardened. All plates were incubated for 48 hours at 37°C. After incubation, the plates were observed for revertant colonies and the colonies were counted and recorded.

The five concentration of test material used in this test were:

1000 ug/plate
500
100
10
1

All concentrations were tested in triplicate against the following strains:

TA 1535
TA 1537
TA 1538
TA 98
TA 100

Table II presents the positive controls used in this study, their test concentration and their specific mutagenic action observed on the tester strain.

Spontaneous revertant controls consisting of the indicator strains and solvent were also included. The solvent and the highest dose level of the test material were checked for sterility by adding 0.1 ml of each to 2 ml of top agar without the test organisms and pouring the entire contents over minimal-glucose agar plates. All plates were then incubated 48 hours at 37°C.

D. Plate Assay for the Detection of Mutagens Requiring Metabolic Activation:

1. Method of Induction and Preparation of Liver Enzymes

The polychlorinated biphenyl mixture, Aroclor 1254, was used for the non-specific induction of liver enzymes. Adult, male Sprague-Dawley rats were given a series of four consecutive, daily I.P. injections of Aroclor 1254 (diluted in corn oil to a concentration of 200 mg/ml). Total dosage for each rat was 500 mg/kg. On the fifth day post injection, all animals were sacrificed by CO₂ asphyxiation. Using aseptic techniques, the liver was immediately dissected from each rat.

VII. METHODS (Continued)

2. Preparation of S-9 Cell Fraction of Liver Homogenates

After dissection, the livers were placed in beakers (pre-weighed) and the weights recorded. The livers were washed with approximately 1 ml homogenizing buffer (0.15 M KCl and 0.05 M Tris-(hydroxymethyl) aminomethane) per gram wet weight, minced with sterile scissors, and three volumes of buffer per gram liver were added. The livers were then homogenized using a motor driven Potter-Elvehjem homogenizing unit at 4°C. The homogenate was centrifuged for 10 minutes at 9000 g (8700 rev/min in SS-34 head of Sorvall RC-5) and the supernatant, designated as S-9 fraction, was decanted, dispensed in 2 ml aliquots, and stored frozen at -80°C until required for testing. All steps were at 0 - 4°C using cold, sterile solutions and glassware. Protein concentration of each bulk preparation of S-9 was determined according to the method of Lowry, et. al. (2).

3. S-9 Fraction Standardization

Each S-9 preparation was diluted with homogenizing buffer to a protein concentration of 10 mg protein/ml S-9. The diluted S-9 preparation was then titrated over a range of concentration (10-150 ul S-9/ml S-9 mix) with 2-aminoanthracene, to determine the optimum amount of S-9 for use in the general screening procedure. The concentration range of S-9 was found to be 50 to 100 ul S-9/ml of S-9 mix (500-1000 ug protein/1 ml S-9 mix).

4. Preparation of S-9 mix

The following is a list of all components and their concentrations used in S-9 buffer:

<u>Component</u>	<u>Final Concentration/ml S-9 mix</u>
MgCl ₂	0.8 mM
KCl	0.33 mM
Glucose-6-phosphate	0.5 mM
NADP	0.4 mM
NaH ₂ PO ₄ . 7 H ₂ O, pH 7.4	100 mM

S-9 buffer was prepared from refrigerated stock solutions of 0.2 M phosphate buffer, 0.4 M MgCl₂, and 1.65 M KCl each day assays were to be conducted. The S-9² was thawed on the day of assay and added to the S-9 buffer (100 ul S-9/1 ml S-9 buffer) in order to prepare fresh S-9 mix. The entire S-9 mix was then passed through a 0.45 um disposable Nalgene filter unit for sterilization and then stored at 4°C until use on that same day.

VII. METHODS (Continued)

5. Activated Plate Assay:

All metabolically activated plate assays were conducted as described above in Section C (Plate Assay for the Detection of Direct Acting Mutagens), the only modification being the addition of 0.5 ml of S-9 mix to each tube of top agar immediately before vortexing and pouring. All test concentrations and test strains remained the same as in Section C.

Table III presents the positive controls used in the activation study, their concentrations, and their specific mutagenic action observed on the indicator strain.

Spontaneous revertant controls consisting of the indicator strains, solvent system and S-9 mix were also included. The solvent, S-9 mix, and highest dose level of the test material were checked for sterility by adding 0.1 ml (0.5 ml in the case of S-9 mix) of each to 2 ml of top agar without the test organisms and pouring the entire contents of each tube over minimal-glucose agar plates. All plates were then incubated 48 hours at 37°C.

E. Criteria

The following criteria were used in the evaluation and reporting of the mutagenic potential of the test material:

1. the spontaneous revertant levels for each strain when used in either the direct plate assay or the activated plate assay must be within the acceptable limits as defined by the historical data shown in Table VI,
2. all sterility controls must be negative,
3. all positive controls must demonstrate that the indicator strains are functional with known mutagens as evidenced by an increase of at least three times the number of revertant colonies per plate as the spontaneous revertant controls.
4. To be considered positive for mutagenic activity, the test material should exhibit a dose response effect (increasing numbers of revertant colonies with increased amounts of the test sample),

VII. METHODS (Continued)

5. for strains TA 1535, TA 1537, TA 1538, and TA 98, the test sample should produce a positive dose response over the concentrations with the lowest increase in revertants/plate greater than or equal to 3x the solvent control value or the S-9 fraction control value, as applicable, to be considered mutagenic, and
6. to be considered mutagenic for strain TA 100, the test sample should produce a positive dose response over three concentrations with at least one dose producing an increase in revertants/plate greater than or equal to 3.5x the solvent control value or the S-9 fraction control value, as applicable.

Criteria (1), (2) and (3) above must be met before the results of testing conducted with any test sample can be considered valid. To be considered mutagenic, criteria (4) and (5) or (6) must also be met.

VIII. RESULTS AND DISCUSSION

Test article IC-2 Lot #91749243 was tested in the Salmonella/Microsomal Mutagenicity Plate Incorporation Assay at doses of 1 ug, 10 ug, 100 ug, 500 ug, and 1000 ug/plate, in triplicate with five tester strains of Salmonella both without metabolic activation and with S-9 metabolic activation. The results are shown in Tables IV and V. Under each strain in the tables is a column of numbers to the left which are the revertant colony counts, and to the right are shown the mean and standard deviation.

Results of the nonactivation direct mutagenicity assay are shown in Table IV. There was no statistically significant increase in mean numbers of revertant colonies above the mean for solvent control plates at any dose tested with any of the five Salmonella tester strains.

Results of the assay with S-9 metabolic activation are shown in Table V. There was no statistically significant increase in mean numbers of revertant colonies above the mean for S-9 control plates at any dose tested with any of the five Salmonella tester strains.

VIII. RESULTS AND DISCUSSION (Continued)

Tables II and III list the positive controls used for direct nonactivation and metabolic activation assays, respectively, and the results are shown in Tables IV and V. All positive controls generated mean numbers of revertant colonies at least three fold greater than the mean for solvent and S-9 negative controls. For each strain, the numbers of revertant colonies in negative control plates were within acceptable limits as defined by the historical data for spontaneous revertants as shown in Table VI. All sterility controls were negative. All of the criteria for a valid test were thus fulfilled.

Results of tests for toxicity of Sample IC-2 Lot #91749243 are shown in Table I. Toxicity was observed at doses of 5000 ug and 10,000 ug/plate for strain TA 1537 and at doses of 5000 ug and 10,000 ug/plate for strain TA 100.

CONCLUSIONS

Under conditions of this study, no mutagenic activity was demonstrated for test article IC-2 Lot #91749243 in either the direct or S-9 - activated Salmonella mutagenesis assays.

IX. STATISTICAL ANALYSIS OF TEST DATA

Least squares linear regression analysis was used to compute the "best fit" regression line of dose response on dose level for the test sample. These regression lines represent the strength of the dose response obtained against each bacterial indicator strain. For each response line (Table VII)

$$y = mx + b,$$

the sample regression coefficient, m , was tested for a statistically significant deviation from the value zero (0) which would indicate mutagenic activity (3). The null hypothesis and the alternative hypothesis are stated below:

H_0 : $m = 0$, the data do not suggest mutagenic activity

H_A : $m > 0$, the data suggest increasing mutagenic activity
dose

Significance level = 0.05

TABLE IDETERMINATION OF SAMPLE TOXICITY TO S. typhimuriumStrains TA 1537 and TA 100

<u>Sample</u>	<u>Concentration (ug/plate)</u>	<u>Percent of Growth Inhibition</u>	
		<u>TA 1537</u>	<u>TA 100</u>
IC-2	10,000	100	100
Lot #91749243	5,000	100	75
	1,000	0	0
	100	0	0
	10	0	0
Controls:			
DMSO	0.1 ml/plate	0	0
9-AA	100 ug/plate	0	-
MNNG	5 ug/plate	-	0

TABLE II
POSITIVE CONTROL USED IN THE DIRECT MUTAGENESIS ASSAY

<u>Indicator Strain</u>	<u>Positive Control Chemical</u>	<u>Conc. (ug/plate)</u>	<u>Solvent</u>	<u>Probable Mutagenic Activity</u>
TA 1535	MNNG	5.0	DMSO	Base-pair Substitution
TA 1537	9-AA	100.0	DMSO	Frameshift Mutation
TA 1538	2-NF	0.5	DMSO	Frameshift Mutation
TA 98	2-NF	0.5	DMSO	Frameshift Mutation
TA 100	MNNG	5.0	DMSO	Base-pair Substitution

TABLE IIIPOSITIVE CONTROLS USED IN THE METABOLIC ACTIVATION MUTAGENESIS ASSAY

<u>Indicator Stain</u>	<u>Positive Cont. of Mutagen</u>	<u>Conc. (ug/plate)</u>	<u>Solvent</u>	<u>Probable Mutagenic Activity</u>
TA 1535	2-AA	2.5	DMSO	Base-pair Substitution
TA 1537	2-AA	2.5	DMSO	Frameshift Mutation
TA 1538	2-AA	2.5	DMSO	Frameshift Mutation
TA 98	2-AA	2.5	DMSO	Frameshift Mutation
TA 100	2-AA	2.5	DMSO	Frameshift Mutation

TABLE IV

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITHOUT METABOLIC ACTIVATION ON SAMPLE IC-2

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS									
		TA 1535		TA 1537		TA 1538		TA 98		TA 100	
DMSO (SOLVENT CONTROL)		20	17.7	8	6.7	13	14.0	53	57.3	130	133.0
		19 ±	3.2	7 ±	1.5	19 ±	4.6	63 ±	5.1	136 ±	3.0
		14		5		10		56		133	
MNNG (POSITIVE CONTROL)	5 ug	1134	1339.3							1613	1461.3
		1340 ±	205.0							1302 ±	155.6
		1544								1469	
2-NF (POSITIVE CONTROL)	0.5 ug					769	805.3	606	676.3		
						819 ±	31.8	739 ±	66.8		
						828		684			
9-AA (POSITIVE CONTROL)	100 ug			330	404.0						
				359 ±	104.1						
				523							
SAMPLE IC-2	1000 ug	20	19.7	8	5.3	21	17.3	40	41.0	136	131.0
		14 ±	5.5	6 ±	3.1	15 ±	3.2	40 ±	1.7	115 ±	14.2
		25		2		16		43		142	
SAMPLE IC-2	500 ug	22	20.0	10	6.0	18	21.7	59	47.3	111	125.0
		19 ±	1.7	3 ±	3.6	23 ±	3.2	47 ±	11.5	120 ±	17.1
		19		5		24		36		144	
SAMPLE IC-2	100 ug	21	25.0	9	9.7	13	15.7	62	56.0	136	130.7
		19 ±	8.7	7 ±	3.1	16 ±	2.5	57 ±	6.6	117 ±	11.9
		35		13		18		49		139	
SAMPLE IC-2	10 ug	26	26.0	6	4.7	22	15.7	57	58.3	139	126.7
		23 ±	3.0	6 ±	2.3	15 ±	6.0	43 ±	16.0	125 ±	11.6
		29		2		10		75		116	
SAMPLE IC-2	1 ug	20	18.7	2	4.7	21	15.7	77	70.0	113	127.3
		22 ±	4.2	2 ±	4.5	14 ±	4.7	58 ±	10.4	128 ±	14.0
		14		10		12		75		141	

TABLE V
RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITH METABOLIC ACTIVATION ON SAMPLE IC-2

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS									
		TA 1535		TA 1537		TA 1538		TA 98		TA 100	
DMSO (SOLVENT CONTROL)		20	17.7	8	6.7	13	14.0	53	57.3	130	133.0
		19 ± 3.2		7 ± 1.5		19 ± 4.6		63 ± 5.1		136 ± 3.0	
		14		5		10		56		133	
B-9 FRACTION (NEGATIVE CONTROL)	50 ul	9	12.7	9	8.7	19	25.0	71	72.0	157	151.3
		13 ± 3.5		8 ± 0.6		24 ± 6.6		58 ± 14.5		141 ± 9.0	
		16		9		32		87		156	
P-AA (POSITIVE CONTROL)	2.5 ug	122	197.0	224	223.3	917	1204.7	1677	1802.7	1251	1650.7
		209 ± 69.8		258 ± 35.0		1264 ± 233.7		1834 ± 113.3		1829 ± 346.8	
		260		188		1403		1897		1872	
SAMPLE IC-2	1000 ug	11	12.7	2	2.7	24	21.3	47	51.3	120	110.0
		21 ± 7.6		1 ± 2.1		15 ± 5.5		44 ± 10.2		96 ± 12.5	
		6		5		25		63		114	
SAMPLE IC-2	500 ug	16	12.3	2	3.7	22	22.0	63	65.3	131	131.7
		11 ± 3.2		5 ± 1.5		22 ± 0.0		79 ± 12.7		109 ± 23.0	
		10		4		22		54		155	
SAMPLE IC-2	100 ug	18	15.3	7	7.7	20	23.3	72	75.0	119	124.7
		9 ± 5.5		6 ± 2.1		19 ± 6.7		69 ± 7.9		127 ± 4.9	
		19		10		31		84		128	
SAMPLE IC-2	10 ug	19	14.7	4	5.0	16	18.0	58	70.3	133	126.0
		14 ± 4.0		5 ± 1.0		21 ± 2.6		83 ± 12.5		116 ± 8.9	
		11		6		17		70		129	
SAMPLE IC-2	1 ug	18	17.0	3	3.7	29	23.7	78	74.7	120	132.3
		16 ± 1.0		4 ± 0.6		18 ± 5.5		72 ± 3.1		152 ± 17.2	
		17		4		24		74		125	

TABLE VI

HISTORICAL BACKGROUND OF SPONTANEOUS REVERTANT LEVELS FOR THE BACTERIAL
INDICATOR STRAINS*

<u>Strain</u>	<u>Mean</u> <u>(# Revertants/Plate)</u>	<u>Standard</u> <u>Deviation</u>	<u>Range</u> <u>(# Revertants/Plate)</u>
A. Plate Assay			
Without Metabolic Activation System			
TA 1535	16.9	7.5	2-43
TA 1537	5.9	2.4	2-15
TA 1538	12.4	3.6	5-23
TA 98	21.5	7.5	9-60
TA 100	143.4	44.5	66-318
B. Plate Assay			
With Metabolic Activation System			
TA 1535	14.5	5.2	5-37
TA 1537	7.3	2.3	2-11
TA 1538	21.7	4.9	12-32
TA 98	33.1	8.4	16-78
TA 100	159.7	51.4	73-407

*All data were derived from all tests conducted within this laboratory and compiled to the present.

TABLE VII

ESTIMATED LEAST SQUARES REGRESSION EQUATION AND STUDENT'S - t VALUES FOR DETERMINING THE STRENGTH OF THE DOSE RESPONSE

Sample	Bacteria Indicator Strain	Regression $y = mx + b$ (Response=Slope x Dose + Intercept)	Student's-t value for Null Hypothesis (H_0 : the data do not suggest mutagenic activity)	Accept H_0 / Reject H_0 (S.L.)* ⁰
<u>IC-2</u>				
Lot #91749243				
A. Test without metabolic activation				
	TA 1535	NA**		
	TA 1537	NA**		
	TA 1538	NA**		
	TA 98	NA**		
	TA 100	NA**		

B. Test
with metabolic
activation

TA	1535	NA**
TA	1537	NA**
TA	1538	NA**
TA	98	NA**
TA	100	NA**

Significance Levels

df 0.05 0.01 0.001

Student - t Critical Values:

*S.L. = Significance Level

**NA = Not applicable; no mutagenic response observed for this strain.

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XI. REFERENCES

1. Ames, B. N., J. McCann, and E. Yamasaki. Mutation Research, 1975, 31: 347-364.
2. Lowry, O. H., N. J. Rosenbrough, A. L. Fair, and R. J. Randall. J. Biol. Chem., 1951, 193: 265-275.
3. Snedecor, G. W. and W. G. Cochran. Statistical Methods, 6th ed. Iowa State University Press, 1967, p. 153.

XII. ABBREVIATIONS

9-AA.....	9-Aminoacridine
2-AA.....	2-Aminoanthracene
CO ₂	Carbon dioxide
DMSO.....	Dimethylsulfoxide
DNA.....	Deoxyribonucleic acid
g.....	Gram
I.P.....	Intraperitoneal
KCl.....	Potassium chloride
kg.....	Kilogram
mg.....	Milligram
MgCl ₂	Magnesium chloride
ml.....	Milliliter
mm.....	Millimeter
MNNG.....	N-methyl-N-nitro-N-nitrosoguanidine
Na ₂ HPO ₄ · 7H ₂ O.....	Sodium phosphate, Dibasic, 7-hydrate
NaCl.....	Sodium chloride
NADP.....	Nicotinamide adenine dinucleotide phosphate
NaH ₂ PO ₄ · H ₂ O.....	Sodium phosphate, Monobasic
2-NF.....	2-Nitrofluorene
nm.....	Nanometer
O.D.....	Optical Density
rev.....	Revolutions
S-9.....	Supernatant obtained from 9000 x g centrifugation of a liver homogenate
u.....	Micro
ug.....	Microgram
ul.....	Microliter



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STUDY REVIEW RECORD

Reference: 81-0960-21

Date of Study Initiation: July 16, 1981

Date of Study Completion: July 23, 1981

Study Director: George C. Lavelle, Ph.D.

Project Leader: Mark Entrup, B.S.

Location of Specimen Storage: N/A

Disposition of Remaining Test Material: At the conclusion of a test program, any unused portion of each sample used will be stored at Hill Top Research, Inc. No materials will be maintained longer than six months after the completion of the study unless the client notifies Hill Top Research, Inc. New drugs are exempt from the above procedure. They will be retained or returned to the client.

Location of Raw Data: Hill Top Research, Inc.
P. O. Box 138
Miamiville, Ohio 45147

Location of Final Report: Hill Top Research, Inc.

Quality Assurance Unit Statement

Date(s) Study Inspected: _____

Date Report Reviewed: September 11, 1981

Date(s) Findings Reported to Management: _____

Date(s) Findings Reported to Study Director: _____

Deanna J. J. J.
Quality Assurance Auditor

September 11, 1981
Date

George C. Lavelle
Quality Assurance Director

9/17/81
Date

ACUTE TOXICITY OF IC-2 TO
BLUEGILL (Lepomis macrochirus).

TOXICITY TEST REPORT
SUBMITTED TO
THE BASF WYANDOTTE CORPORATION
WYANDOTTE, MICHIGAN

REPORT #BW-81-8-969

EG&G, Bionomics
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
August, 1981

INTRODUCTION

The purpose of this study was to estimate the acute toxicity of IC-2 to bluegill (Lepomis macrochirus) under static conditions. A 96-hour definitive test was conducted from 4-8 August 1981 at the Aquatic Toxicology Laboratory of EG&G, Bionomics, Wareham, Massachusetts. All raw data generated are stored at the above location.

MATERIALS AND METHODS

Unless otherwise stated, procedures used in this acute toxicity test followed those described in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA, 1975) and the protocol entitled "EG&G, Bionomics Protocol for Freshwater Static Acute Toxicity Tests with Fish." Values are reported to different levels of significance depending on the accuracy of the measuring devices involved in any one process.

The IC-2 (c-amine; Intermediate chemical, lot #91749243; 7/9/81), an off-white colored powder, tested as 100% active ingredient, was received from the BASF Wyandotte Corporation, Wyandotte, Michigan on 14 July 1981. Nominal test concentrations are reported as milligrams of IC-2 per liter of test solution (mg/l).

The bluegill (Bionomics lot #81D7) were obtained from a commercial fish supplier in Connecticut and held in a 500-l fiberglass tank under a photoperiod of 16 hours light and 8 hours darkness. All fish were fed a dry pelleted food, ad libitum, daily except during the 48 hours prior to testing. There was 0.1% mortality in the test fish population during this 2 day period (Daily Record of Fish Holding Conditions). The well water which flowed into this tank was characterized as having total hardness and alkalinity ranges as calcium carbonate (CaCO₃) of 26-30 mg/l and 22-26 mg/l, respectively, and a specific conductance of 125-130 micromhos per centimeter (µmhos/cm) (Weekly Gravity Feed Tank Water Quality Analysis Logbook). Other parameters monitored in the holding tank were a pH range of 6.2-6.7, a dissolved oxygen (DO) range of 87-95% of saturation and a flow rate of 10-11 tank volume replacements/day (Weekly Record of Fish Holding Water Characteristics). Test fish were maintained under these conditions for a minimum of 14 days. The temperature in the holding tank was 19-22°C during this 14 day period (Daily Record of Fish Holding Conditions). The specific conductance was measured with a YSI Model #33 salinity-conductivity-temperature meter and probe, the pH was measured with an Instrumentation Laboratory Model #175 pH meter and combination electrode, the DO was measured with a YSI Model #57 dissolved oxygen meter and probe and the temperature was measured with a Brooklyn alcohol thermometer. Total hardness and alkalinity were measured according to APHA et al. (1975).

The definitive test was conducted in 19.6- ℓ glass jars which contained 15 ℓ of test solution. The dilution water used was soft water reconstituted from deionized water according to recommended procedures (U.S. EPA, 1975). This water had a total hardness and alkalinity as CaCO₃ of 41 mg/ ℓ and 31 mg/ ℓ , respectively, a pH of 7.4 and a specific conductance of 190 μ mhos/cm (Reconstituted Water, Water Quality Analysis).

Test mixtures were prepared by adding the desired amount of IC-2 directly to each test jar containing 15 ℓ of diluent water. The test jars were then mixed for 5 minutes with an Eberbach Lab-Stir equipped with a stainless steel shaft and blade.

A control jar containing the same dilution water as used in the exposure jars, but containing no IC-2, was maintained. All test solution temperatures were controlled by a system designed to maintain temperatures at $22 \pm 1^{\circ}\text{C}$. Test solutions were not aerated. The photoperiod during testing was the same as that provided during acclimation.

Ten bluegill with a mean (range, N=30) wet weight and total length of 0.34(0.14-0.59) grams and 31(24-42) millimeters (Fish Weights and Lengths Log) were randomly distributed to each test jar within 15 minutes after the test solutions had been prepared

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Mortalities were recorded and removed from each test jar and biological observations of the fish and observations of the physical characteristics of the test solutions were made and recorded at 0, 24, 48, 72 and 96 hours of exposure. The pH and DO concentrations were measured at 0, 24, 48 and 96 hours in the control and the high, middle and low test concentrations. The temperature was measured in the control jar at 0, 24, 48, 72 and 96 hours of exposure.

The concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate 24-, 48-, 72- and 96-hour median lethal concentrations (LC50) and 95% confidence intervals. The LC50 is defined as the concentration (nominal or measured) of the test compound in dilution water which caused mortality of 50% of the test animal population at the stated exposure interval. The computer program utilized (Stephan, 1978, personal communication) estimated LC50 values using one of three statistical methods in the following order of preference: moving average angle analysis, probit analysis, binomial probability. The method selected was determined by the characteristics of the data base (i.e. presence or absence of test concentrations causing mortality of 100% of the test animal population, test concentrations causing mortality of a partial number of animals in the population, etc.). The computer program scanned the data base, identified the most preferred statistical method and performed the analysis.

RESULTS

The 96-hour LC50 (and 95% confidence interval) for bluegill exposed to IC-2, estimated by binomial probability, was 170 (130-220) mg/l.

Table 1 summarizes the 24-, 48-, 72- and 96-hour LC50's and 95% confidence intervals and states the no discernible effect concentration through 96 hours. The no effect concentration is defined as the highest concentration tested at which there were no mortalities or observed behavioral and physical abnormalities (i.e. loss of equilibrium, fish at surface, darkened pigmentation).

The temperature in the control jar was $22 \pm 0^{\circ}\text{C}$ during exposure. The pH and dissolved oxygen concentrations measured during the toxicity test are presented in Table 2. Table 3 presents the nominal test concentrations, the corresponding percentage mortalities and the observations made.

LITERATURE CITED

APHA, ANWA, WPCF. 1975. Standard methods for the examination of water and wastewater. 14th Edition, Washington, D.C. 1193 pp.

Stephan, Charles. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication.

U.S. EPA. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecological Research Series (EPA-660/3-75-009), 61 pp.

Table 1. The 24-, 48-, 72- and 96-hour LC50 values and 95% confidence intervals for bluegill (Lepomis macrochirus) exposed to IC-2.

	LC50 (mg/l) ^a		No discernible effect concentration through 96 hours (mg/l)
	48 hour	72 hour	96 hour
170 (130-220) ^b	170 (130-220)	170 (130-220)	130

^a Estimated by binomial probability.

^b 95% confidence interval.

Table 2. The pH and DO concentrations measured during a 96-hour toxicity test with IC-2 and bluegill (Lepomis macrochirus).

	Nominal concentration (mg/l)	0 hour	24 hour	48 hour	96 hour
pH	600	3.2	2.9	3.0	2.4
	220	3.5	3.5	3.4	3.3
	46	6.3	6.3	6.4	6.3
	control	7.2	6.8	6.8	6.5
DO (mg/l)	600	8.3(94)	8.6(98)	8.1(92)	7.2(82)
	220	8.5(97)	8.3(94)	4.9(56)	6.4(73)
	46	8.7(99)	5.6(64)	4.4(50)	3.0(34)
	control	8.7(99)	5.9(67)	4.4(50)	3.8(43)

^a % of saturation at 22°C.

Table 3. Concentrations tested and corresponding percentage mortalities of bluegill (Lepomis macrochirus) exposed to IC-2 for 24, 48, 72 and 96 hours.

Nominal concentration ^a (mg/l)	% mortality			
	24 hour	48 hour	72 hour	96 hour
600	100 ^b	100 ^b	100 ^b	100 ^b
360	100 ^b	100 ^b	100 ^b	100 ^b
220	100 ^b	100 ^b	100	100
130	0	0	0	0
78	0	0	0	0
46	0	0	0	0
control	0	0	0	0

^a Undissolved test material was present on the bottom of the test vessels at 0 hour.

^b Undissolved test material was present on the bottom of the test vessel.

SUBMITTED BY:

EG&G, Bionomics
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August, 1981

PRINCIPAL INVESTIGATOR:

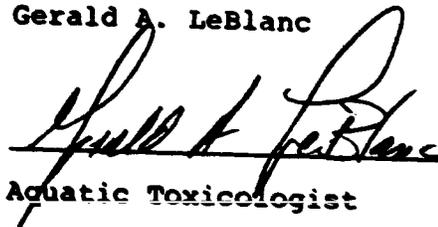
Joseph V. Sousa



Aquatic Biologist

STUDY DIRECTOR:

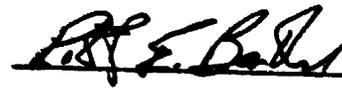
Gerald A. LeBlanc



Aquatic Toxicologist

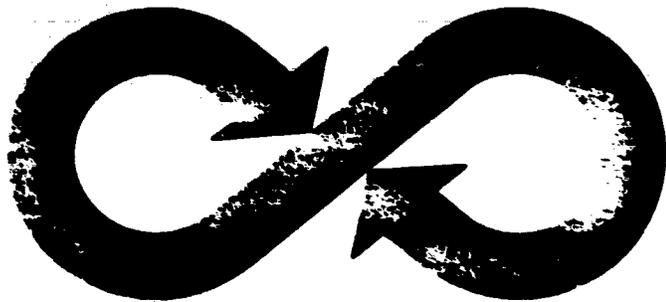
DATA AUDITED BY:

Robert E. Bentley



Director, Quality Assurance Unit

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Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO.: 82-7564

**AN ACUTE INHALATION TOXICITY STUDY
OF C-AMINE IN THE RAT**

**Submitted to: Hill Top Research Inc.
P.O. Box 42501
Cincinnati, Ohio 45242**

Attention: Dr. David Conine

Date: June 29, 1982

0 0 5 1

I. GENERAL

A one-hour inhalation exposure was performed using Sprague-Dawley derived (CD®) rats to determine the acute toxicity of C-Amine. The test material, a greyish-white powder was labelled "IC-Z 014325 91749243 MS," and was received on March 9, 1982. The purity of the material was >98%. All testing was conducted at Bio/dynamics Inc., P.O. Box 43, Mettlers Road, East Millstone, New Jersey, 08873 where the protocol, data, and report will be stored in the archives.

II. EXPERIMENTAL

The test material was press-packed using a Carver Hydraulic Press at a pressure of 100 pounds per square inch (psi) into the cylinder of a Wright dust-feed mechanism. Dry air, at a flow rate of 20 liters per minute (lpm), was passed through the dust-feed mechanism. The resultant dust-laden airstream was directed, undiluted, into a 100 liter (l) Plexiglas® exposure chamber containing the test animals. One cylinder was required to complete the exposure which lasted one hour.

The generation apparatus and test material were weighed before and after the exposure. The difference in weight represented the total amount of test material delivered into the chamber; this, divided by the total volume of air delivered yielded the nominal exposure concentration.

A wet-bulb/dry-bulb hygrometer was used to monitor chamber air temperature and relative humidity which were recorded at an hourly interval during the exposure.

The test group consisted of five male and five female Sprague-Dawley derived (CD®) rats obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts on March 9, 1982. The animals were held for 21 days pre-exposure during which time animals considered suitable for test material

II. EXPERIMENTAL (Cont.)

exposure were sorted into groups on a random basis and individually identified by eartag. The animals were housed individually in elevated, stainless steel and wire mesh cages and provided with city tap water (Elizabethtown Water Co.) and pelleted food (Purina Rodent Laboratory Chow - 5001) ad libitum during the non-exposure period. No food or water was available during the exposure.

On the day of exposure (Day 0 - March 30, 1982) the pre-exposure body weights were in the ranges 244-298 grams (males) and 216-228 grams (females). The animals were observed for abnormal signs before exposure, every fifteen minutes during the exposure period, upon removal from the chamber, for four hours post-exposure, and daily thereafter for 14 days. Individual body weights for all rats were recorded on Day 0 (prior to exposure) and on Days 1, 2, 4, 7, and 14. On Day 14 (April 13, 1982), all rats were exsanguinated under ethyl ether anesthesia and gross necropsy examinations were performed. No tissues were saved.

III. RESULTS AND DISCUSSION

During the exposure, a total of 13.76 grams of test material was delivered in a total volume of 1200 liters of air, yielding a nominal exposure concentration of 11 milligrams per liter (mg/l). The mean chamber temperature was 69°F and the mean relative humidity was 84%.

No rats died during the exposure or subsequent 14-day observation period.

Most rats appeared unaffected during the exposure period; some had eyes partially closed after 15 minutes of exposure.

After removal from the exposure chamber, all rats exhibited lacrimation for up to four hours post-exposure, which had not abated by the time of the observation on the next day. Some rats exhibited signs of matted and wet fur, salivation, red, dried-red and mucoid nasal discharge; all these signs

0 0 6 3

III. RESULTS AND DISCUSSION (Cont.)

completely abated by the observation on the next day, except the mucoid nasal discharge (Table 1).

Lacrimation was seen in most animals (predominantly males) during the 14-day observation period. The frequency of this observation remained relatively constant through Day 11 but afterwards it reduced in frequency. This is interpreted as a treatment-related effect. Other signs (mucoid, red, or dried red nasal discharge and dry rales) were seen infrequently in isolated rats and did not appear associated with exposure to the test material (Table 2).

Although small, transient weight-losses were seen in all rats (due to exposure procedure), the body weights recovered to pre-exposure values in males by Day 4 and in females by Day 7. Body weight increments in the second week were within the limits of normal expectation for both sexes, and thus, were not indicative of a response to exposure (Table 3).

At necropsy, 4 male and 1 female rats showed foci or areas of lung discoloration and/or surface irregularities. These are common pathological findings in Sprague-Dawley rats. However, in males it may be related to exposure due to the higher incidence than is normally encountered. In addition, one female had both ovaries surrounded by a capsule filled with clear fluid (Table 3).

0 0 6 4

IV. CONCLUSION

A one-hour acute inhalation exposure to C-Amine at a nominal concentration of 11 milligrams per liter did not produce mortality; however, an increase in lacrimation (predominantly in males) indicated a response to exposure. Body weights did not reveal effects from exposure. Necropsy observations of lung discoloration, mottling, and surface irregularities may be indicative of a response to treatment in males.

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Study Director, Inhalation Toxicology

6/29/82
Date

James B. Terrill
James B. Terrill, Ph.D.
Director of Inhalation Toxicology

6-29-82
Date

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Vice-President of Toxicology

6/29/82
Date

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- Sandra Minnella, B.S.; Study Monitor, Inhalation

0 0 6 5

82-7564

Table 1
An Acute Inhalation Toxicity Study
of C-Amine in the Rat

Summary of Exposure and Post-Exposure Observations

	Pre- last	15	30	45	60	*T	120	180	240	300
<u>SECRETORY</u>										
Lacrimation						10	10	10	10	10
Mucoid nasal discharge						1	6	6	2	5
Dried red nasal discharge						3	3	4	1	
Salivation						1				
Dried red material around facial area						2	3	1		
<u>GENERAL</u>										
Eyes partially closed			some	some	some					
Fur wet						7	5	2	1	
Fur matted						3	2	3	2	2
No observed abnormalities										all

*T - Animals observed upon removal from the chamber.

82-7564

Table 2
 An Acute Inhalation Toxicity Study
 of C-Amine in the Rat
 Summary of 14-Day Post-Exposure Observations

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<u>SECRETORY</u>														
Lacrimation	9	7	7	5	5	7	8	6	8	7	2	3	4	3
Mucoid nasal discharge	1		1		6	3	3	3	1				2	
Red nasal discharge			1				1							1
Dried red nasal discharge	1	1	1		1			1	1		2			
<u>RESPIRATORY</u>														
Dry rales				2					1					
Moist rales														
No observed abnormalities	1	3	2	4	5	2	2	3	1	3	6	7	6	7

Table 3
 An Acute Inhalation Toxicity Study
 of C-Amine in the Rat

82-7564

Individual Body Weights and Necropsy Findings

Animal Number	Sex	Body Weight (g) on Day:				Necropsy Findings*		
		0	2	4	14			
1436	M	294	289	291	304	315	342	Lungs - all lobes, scattered grey foci (approx. 0.1 cm. diam.).
1437	M	283	276	282	296	316	347	Lungs - all lobes, scattered dark brown foci (<0.2 cm. diam.).
1438	M	244	239	245	253	267	292	N.O.A.
1439	M	298	294	299	314	335	375	Lungs - all lobes, slightly mottled red and grey; L. lobe exhibits slight irregular surface.
1440	M	271	262	266	276	294	326	Lungs - all lobes, scattered red foci (approx. 0.1 cm. diam.).
1936	F	228	225	225	225	230	242	Lungs - all lobes, mottled red and tan.
1937	F	224	218	218	224	226	242	N.O.A.
1938	F	228	221	218	226	230	236	Ovaries - B. cystic, capsule surrounds body of B. ovaries (approx. 0.5 cm. diam.), filled with clear fluid.
1939	F	216	205	210	212	223	234	N.O.A.
1940	F	223	211	212	217	228	230	N.O.A.

*Key: N.O.A. = no observed abnormalities; L = Left; B = Both.

Phil Webb



Hill Top Research, Inc.

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[BASF Wyandotte Corporation]
 Wyandotte, Michigan 48192
 Attn: Mr. Daniel C. Steinmetz
 Industrial Toxicologist
 L]

INVOICE NO. 014721

INVOICE DATE 7/13/82

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Our Project No.

Project Description

Amount

82-0147-21

Acute Inhalation Study

\$ 990.00

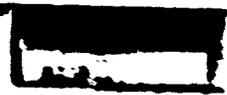
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ABC Preliminary Report #32403

"Determination of Octanol/Water Partition
Coefficient of C-Amine"

Submitted To:

Daniel Steinmetz, Ph.D.
BASF Wyandotte Corporation
491 Columbia Avenue
Holland, Michigan 49423

February 28, 1985

Submitted By: Analytical Bio-Chemistry Laboratories, Inc.
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The Principal Investigator and Study Director of the project for Analytical Bio-Chemistry Laboratories, Inc., was Julie Warren, Chemist II. The Study Director for BASF Wyandotte Corporation was Dr. Daniel Steinmetz. The following ABC personnel assisted with various phases of the study.

<u>Name, Title</u>	<u>Signature</u>	<u>Initials</u>
Julie Warren, Chemist II	<u>Julie Warren</u>	<u>JW</u>
Carol Carlton, Lab Technician I	<u>Carol Carlton</u>	<u>CC</u>
Leanne Forbis, Analyst II	<u>Leanne Forbis</u>	<u>LF</u>
Del Teeter, Chemist I	<u>Del Teeter</u>	<u>DT</u>
Patti Williams, Lab Technician I	<u>Patti Williams</u>	<u>PSW</u>

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SUMMARY

An octanol/water partition coefficient (P) determination was conducted on Red Lake C-Amine at three pH levels.

Duplicate test solutions were prepared in aqueous buffered solutions at 0.1% and 0.01% w/v for pH 7 and pH 9, and 0.05% and 0.005% w/v for pH 5. Five milliliter aliquots of these solutions were partitioned with 5 ml of octanol and aliquots of the octanol and water phases were drawn and analyzed for their C-Amine concentration. The concentration in the octanol layer at all 3 pH levels was below the minimum detectable limit of the analytical method.

The P value for each equilibrium system was calculated based on the minimal detectable limit for C-Amine in octanol and the high test concentrations of C-Amine in the aqueous phase.

The P value for C-Amine in pH 5, pH 7 and pH 9 is less than 10.

INTRODUCTION

The BASF Wyandotte Corporation contracted Analytical Bio-Chemistry Laboratories, Inc. to conduct an octanol/water partition coefficient (P) study of C-Amine at three pH levels. This study was authorized by Dr. Daniel Steinmetz in a letter dated October 24, 1984 and was conducted from December 3, 1984 to February 12, 1985.

METHODS AND MATERIALS

This study was conducted following the methods outlined in the ABC protocol #A-8003 revised November 20, 1984 for BASF Wyandotte Corporation entitled "Determination of Octanol/Water Partition Coefficients" (Appendix II).

I. Test Compound

C-Amine was received from BASF Wyandotte Corporation on November 8, 1984, a white powder in a vial labeled Red Lake C-Amine, Lot #2406-62, and was stored in the freezer when not in use.

II. Test Water

All test water used in this study was deionized water boiled for 30 minutes and filtered through a 0.22 micron filter. The water was saturated with test octanol before use.

III. Test n-Octanol

All test n-octanol was ACS certified 1-octanol which had been washed with 0.1 N HCl, 0.1 N KOH and water. The octanol was saturated with test water before use.

IV. Test System

The test system consisted of 16 X 125 mm culture tubes with inert (Teflon) lined caps, an Environ Shaker[®] at 23°C ±1°C and an IEC Clinical centrifuge.

V. Test Procedure

Solutions of 0.1% and 0.01% w/v of C-Amine in a pH 7 and pH 9 aqueous buffer, and 0.05% and 0.005% w/v in a pH 5 aqueous buffer were prepared. Duplicate 5 ml aliquots of the pH 7 and pH 9 0.1% and 0.01% test solutions and pH 5 0.05% and 0.005% test solution were added to culture tubes. Five milliliters of n-octanol was then added to each culture tube and capped tightly.

The samples were then shaken for 24 hours at 23°C in an Environ Shaker[®].

The samples were transferred to an environmentally controlled chamber and let stand for 24 hours at 25°C ±1°C.

The water layer was drawn off into 16 X 125 mm culture tubes and centrifuged for 30 minutes.

The octanol layer was then drawn off into culture tubes and centrifuged for 30 minutes. Appropriate dilution of each aqueous and octanol sample were made for HPLC analysis.

VI. HPLC Analysis

The HPLC method for C-Amine was supplied by BASF Wyandotte Corporation Pigments division and was accomplished using a Waters Model 6000-A Solvent Delivery System, a Schoeffel Spectroflow SF-770 U.V. detector, and a Rheodyne Model no. 7125 injector.

The HPLC parameters were as follows.

Column: Aitech 25 cm X 4.6 mm, reverse phase C₁₈ 5 μ
Mobile Phase: 0.005 M NH₄C₂H₃O₂/Methanol 70/30
Flow Rate: 0.8 ml/minutes
Wavelength: 220 nm
Range: 0.02 AUFS
Pressure: 1500 psi
Injection Volume: 20 μl

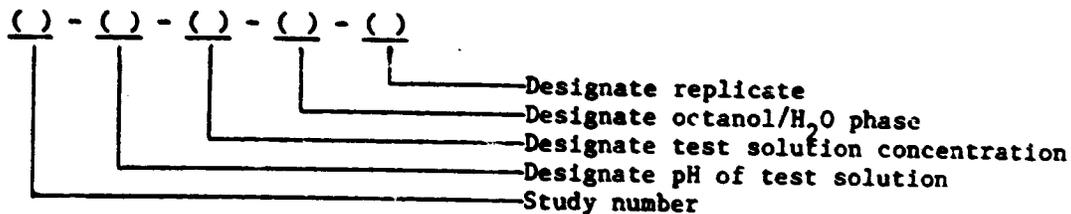
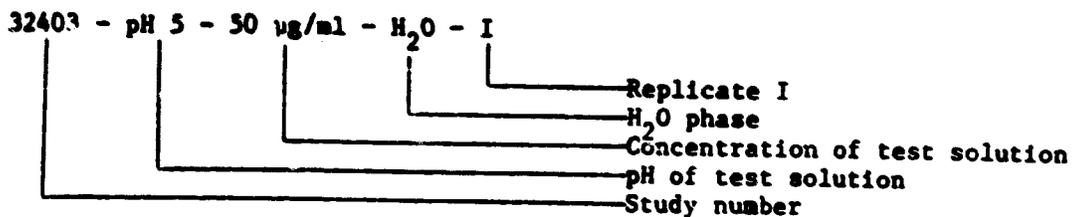
The concentration of C-Amine was determined directly from the standard curve using the linear regression function of a TI-66 calculator. The minimum detectable limit were determined from line equation of the standard curve, as 2X intercept.

Calculation of the partition coefficient was based on the minimum detectable limit of C-Amine in octanol versus the concentration of C-Amine in the aqueous phase.

RESULTS AND DISCUSSION

The octanol/water partition coefficient (P) of the test compound C-Amine was determined by HPLC analysis. Analysis of each test system yielded an approximate P value of less than 10.

TABLE 1: Sample Code.



Study Phases

Octanol = Oct
Water = H₂O

Replicate

I
II

Test Solution

pH 5
pH 7
pH 9

Test Concentration

500 $\mu\text{g/ml}$ / 50 $\mu\text{g/ml}$
1.0 mg/ml / 100 $\mu\text{g/ml}$
1.0 mg/ml / 100 $\mu\text{g/ml}$

TABLE 2: Partition Coefficient of C-Amine.

Sample Code	Concentration Octanol*	Concentration H ₂ O	P	= log P
pH 5-500 µg/ml-I	<0.476	279	<0.001	-6.4
pH 5-500 µg/ml-II	<0.476	277	<0.002	-6.4
pH 5-50 µg/ml-I	<0.476	33.8	<0.014	-4.3
pH 5-50 µg/ml-II	<0.476	32.5	<0.015	-4.2
pH 7-1.0 mg/ml-I	<0.476	953	<0.0005	-7.6
pH 7-1.0 mg/ml-II	<0.476	947	<0.0005	-7.6
pH 7-100 µg/ml-I	<0.476	92.0	<0.005	-5.3
pH 7-100 µg/ml-II	<0.476	93.4	<0.005	-5.3
pH 9-10 mg/ml-I	<2.65	891	<0.003	-5.8
pH 9-10 mg/ml-II	<2.65	966	<0.003	-5.9
pH 9-100 µg/ml-I	<2.65	82.4	<0.032	-3.4
pH 9-100 µg/ml-II	<2.65	73.6	<0.036	-3.3

*Less than values determined from minimum detectable limit.

Quality Assurance Statement for Preliminary Report #32403, Determination of Octanol/Water Partition Coefficient of C-Amine, for Dr. Daniel Steinmetz, BASF Wyandotte Corporation, Holland, Michigan.

In accordance with ABC Laboratories' intent that all studies conducted by our facility meet or exceed the criteria promulgated by the various federal agencies to assure the accuracy and precision of analytical results, the above named report was reviewed and found to be in acceptable form by a member of our Quality Assurance Unit.

A final inspection of all data and records on February 26, 1985, indicated that the report submitted to you is an accurate reflection of the study as it was conducted by ABC Laboratories.

If you should have any questions concerning this statement or the function of our Quality Assurance Unit, please contact the QA Unit at your convenience.

Phillip M. Buckler 2/28/85
Phillip M. Buckler Date
Quality Assurance Officer

Study Compliance Statement for Report #32403, Determination of Octanol/Water Partition Coefficient of C-Amine, for Dr. Daniel Steinmetz, BASF Wyandotte Corporation, Holland, Michigan.

In accordance with ABC Laboratories' intent that all environmental fate tests conducted by our facility follow good laboratory practices, ABC's study director for the above test confirms that the study was conducted in compliance with the U.S. E.P.A. Good Laboratory Practice Standards; Toxic Substances Control (40 CFR 792).

All original raw data will be sent to BASF Wyandotte Corporation with the final report and a copy retained at Analytical Bio-Chemistry Laboratories.

Julie Warren 2/28/85
Julie Warren Date
ABC Study Director