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E I DUPONT DE NEMOURS & CO

Contractor

HASKELL LAB

Document Title

INITIAL SUBMISSION: EMBRYOTOXIC AND TERATOGENIC STUDIES IN RATS WITH INHALED HEXAFLUOROISOPROPANOL (HFIP) (FINAL REPORT) WITH COVER LETTER DATED 032792

Chemical Category

HEXAFLUOROISOPROPANOL



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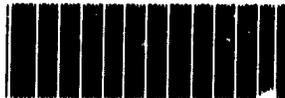
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Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460
Attn: Section 8(e) Coordinator (CAP Agreement)



38920001529

92 MAR 30 AM 8:31

Dear Coordinator:

8ECAP-0025

On behalf of the Regulatee and pursuant to Unit II B.1.b. and Unit II C of the 6/28/CAP Agreement, E.I. Du Pont de Nemours and Co. hereby submits (*in triplicate*) the attached studies. Submission of this information is voluntary and is occasioned by recent changes in EPA's standard as to what EPA considers as reportable information under TSCA Section 8(e). Regulatee's submission of information is made solely in response to the new EPA §8(e) reporting standards¹ and is not: an admission: (1) of TSCA violation or liability; (2) that Regulatee's activities with the study compounds reasonably support a conclusion of substantial health or environmental risk or (3) that the studies themselves reasonably support a conclusion of substantial health or environmental risk.

For Regulatee,

Mark H. Christman
Counsel
Legal D-7010-1
1007 Market Street
Wilmington, DE 19898
(302) 774-6443

¹In particular, most of the reports submitted herein are reproduction/developmental toxicity studies for which the effects noted (reduced birth weight; skeletal defects) occurred at maternally toxic dose levels and which do not support the conclusion that the test compound presents a reproduction/developmental risk. Regulatee notes that reporting this type of information under TSCA §8(e) was announced for the first time in EPA's June 1991 "Reporting Guide".

CONTAINS NO CBI

ATTACHMENT 1

Submission of information is made under the 6/28/91 CAP Agreement, Unit II. This submission is made voluntarily and is occasioned by recent changes in EPA's TSCA §8(e) reporting standard; such changes published for the first time in 1991 and impermissibly applied retroactively. Regulatee's submission of information under this changed standard is not an admission of TSCA violation or liability of that Regulatee's activities with the study compounds reasonably support a conclusion of substantial risk to health or to the environment. Regulatee has historically relied in good faith upon the 1978 Statement of Interpretation and Enforcement Policy criteria for determining whether study information is reportable under TSCA §8(e), 43 Fed Reg 11110 (March 16, 1978). EPA has not, to date, amended this Statement of Interpretation.

Upon CAP registration, EPA provided the Regulatee the June 1, 1991 "TSCA Section 8(e) Reporting Guide". EPA has not indicated that the "Reporting Guide" supersedes the Statement of Interpretation. The "Reporting Guide" considerably lowers the Statement of Interpretation's TSCA §8(e) reporting standard. This is particularly troublesome as the "Reporting Guide" states criteria, which if applied retroactively, expands upon and conflicts with the Statement of Interpretation. Absent amendment of the Statement of Interpretation, the informal issuance of the "Reporting Guide" clouds the appropriate standard by which regulated persons must assess information for purposes of TSCA §8(e).

The following examples of new criteria contained in the "Reporting Guide" that is not contained in the Statement of Interpretation follow:

- even though EPA expressly disclaims each "status report" as being preliminary evaluations that should not be regarded as final EPA policy or intent², the "Reporting Guide" gives the "status reports" great weight

²The 'status reports' address the significance, if any, of particular information reported to the Agency, rather than stating EPA's interpretation of §8(e) reporting criteria. In the infrequent instances in which the status reports contain discussion of reportability, the analysis is invariably quite limited, without substantial supporting scientific or legal rationale.

- as "sound and adequate basis" from which to determine mandatory reporting obligations. ("Guide" at page 20).
- the "Reporting Guide" contains a matrix that establishes new numerical reporting "cutoff" concentrations for acute lethality information ("Guide" at p. 31). Neither this matrix nor the cutoff values therein are contained in the Statement of Interpretation. The regulated community was not made aware of these cutoff values prior to issuance of the "Reporting Guide" in June, 1991.
 - the "Reporting Guide" states new specific definitional criteria with which the Agency, for the first time, defines as 'distinguishable neurotoxicological effects'; such criteria/guidance not expressed in the 1978 Statement of Interpretation.³;
 - the "Reporting Guide" provides new review/ reporting criteria for irritation and sensitization studies; such criteria not previously found in the 1978 Statement of Interpretation/Enforcement Policy.
 - the "Reporting Guide" publicizes certain EPA Q/A criteria issued to the Monsanto Co. in 1989 which are not in the Statement of Interpretation; have never been published in the Federal Register or distributed by the EPA to the Regulatee. Such Q/A establishes new reporting criteria not previously found in the 1978 Statement of Interpretation/Enforcement Policy.

In discharging its responsibilities, an administrative agency must give the regulated community fair and adequate warning to as what constitutes noncompliance for which penalties may be assessed.

Among the myriad applications of the due process clause is the fundamental principle that statutes and regulations which purport to govern conduct must give an adequate warning of what they command or forbid.... Even a regulation which governs purely economic or commercial activities, if its violation can engender penalties, must be so framed as to provide a constitutionally adequate warning to those whose activities are governed.

Diebold, Inc. v. Marshall, 585 F.2d 1327, 1335-36 (D.C. Cir. 1978).

While neither the Statement of Interpretation nor the "Reporting Guide" is a rule, this fundamental principle has been

³ See, e.g., 10/2/91 letter from Du Pont to EPA regarding the definition of 'serious and prolonged effects' as this term may relate to transient anesthetic effects observed at lethal levels; 10/1/91 letter from the American Petroleum Institute to EPA regarding clarification of the Reporting Guide criteria.

applied to hold that agency 'clarification' will not applied retroactively.

...a federal court will not retroactively apply an unforeseeable interpretation of an administrative regulation to the detriment of a regulated party on the theory that the post hoc interpretation asserted by the Agency is generally consistent with the policies underlying the Agency's regulatory program, when the semantic meaning of the regulations, as previously drafted and construed by the appropriate agency, does not support the interpretation which that agency urges upon the court.

Standard Oil Co. v. Federal Energy Administration, 453 F. Supp. 203, 240 (N.D. Ohio 1978), aff'd sub nom. Standard Oil Co. v. Department of Energy, 596 F.2d 1029 (Em. App. 1978):

The 1978 Statement of Interpretation does not provide adequate notice of, and indeed conflicts with, the Agency's current position at §8(e) requires reporting of all 'positive' toxicological findings without regard to an assessment of their relevance to human health. In accordance with the statute, EPA's 1978 Statement of Interpretation requires the regulated community to use scientific judgment to evaluate the significance of toxicological findings and to determining whether they reasonably support a conclusion of a substantial risk. Part V of the Statement of Interpretation urges persons to consider "the fact or probability" of an effect's occurrence. Similarly, the 1978 Statement of Interpretation stresses that an animal study is reportable only when "it contains reliable evidence ascribing the effect to the chemical." 43 Fed Reg. at 11112. Moreover, EPA's Statement of Interpretation defines the substantiality of risk as a function of both the seriousness of the effect and the probability of its occurrence. 43 Fed Reg 11110 (1978). Earlier Agency interpretation also emphasized the "substantial" nature of a §8(e) determination. See 42 Fed Reg 45362, 45363 (1977). [Section 8(e) findings require "extraordinary exposure to a chemical substance...which critically imperil human health or the environment"].

The recently issued "Reporting Guide" guidance requires reporting beyond and inconsistent with that required by the Statement of Interpretation. Given the statute and the Statement of Interpretation's explicit focus on substantial human or environmental risk, whether a substance poses a "substantial risk" of injury requires the application of scientific judgment to the available data on a case-

by-case basis. If an overall weight-of-evidence analysis indicates that this classification is unwarranted, reporting should be unnecessary under §8(e) because the available data will not "reasonably support the conclusion" that the chemical presents a substantial risk of serious adverse consequences to human health.

Neither the legislative history of §8(e) nor the plain meaning of the statute support EPA's recent lowering of the reporting threshold that TSCA §8(e) was intended to be a sweeping information gathering mechanism. In introducing the new version of the toxic substances legislation, Representative Eckhart included for the record discussion of the specific changes from the version of H. R. 10318 reported by the Consumer Protection and Finance Subcommittee in December 1975. One of these changes was to modify the standard for reporting under §8(e). The standard in the House version was changed from "causes or contributes to an unreasonable risk" to "causes or significantly contributes to a substantial risk". This particular change was one of several made in TSCA §8 to avoid placing an undue burden on the regulated community. The final changes to focus the scope of Section 8(e) were made in the version reported by the Conference Committee.

The word "substantial" means "considerable in importance, value, degree, amount or extent". Therefore, as generally understood, a "substantial risk" is one which will affect a considerable number of people or portion of the environment, will cause serious injury and is based on reasonably sound scientific analysis or data. Support for the interpretation can be found in a similar provision in the Consumer Product Safety Act. Section 15 of the CPSA defines a "substantial product hazard" to be:

"a product defect which because of the pattern of defect, the number of defective products distributed in commerce, the severity of the risk, or otherwise, creates a substantial risk of injury to the public."

Similarly, EPA has interpreted the word 'substantial' as a quantitative measurement. Thus, a 'substantial risk' is a risk that can be

quantified, *See*, 56 Fed Reg 32292, 32297 (7/15/91). Finally, since information pertinent to the exposure of humans or the environment to chemical substances or mixtures may be obtained by EPA through Sections 8(a) and 8(d) regardless of the degree of potential risk, §8(e) has specialized function. Consequently, information subject to §8(e) reporting should be of a type which would lead a reasonable man to conclude that some type action was required immediately to prevent injury to health or the environment.

quantified, *See*, 56 Fed Reg 32292, 32297 (7/15/91). Finally, since information pertinent to the exposure of humans or the environment to chemical substances or mixtures may be obtained by EPA through Sections 8(a) and 8(d) regardless of the degree of potential risk, §8(e) has specialized function. Consequently, information subject to §8(e) reporting should be of a type which would lead a reasonable man to conclude that some type action was required immediately to prevent injury to health or the environment.

-7-

ATTACHMENT 2

Submission Summary

0009

CAS # 920-66-1

Chem: Hexafluoroisopropanol (HFIP)

Title: Embryotoxic and Teratogenic Study by Inhalation
in Rats

Date: 3/3/77

Summary of Effects:

- At concentrations of 30 and 200ppm there were no effects on body weight, no changes in behavior or gross pathological changes. Pregnancy was not affected and embryotoxicity was not observed. No major external, visceral or skeletal malformations were observed; however, there was a slight increase in the incidence of bipartite thoracic centra at 200ppm level.

-9-

ATTACHMENT 3

Study Report

**EMBRYOTOKIC AND TERATOGENIC STUDIES IN RATS
WITH INHALED HEXAFLUOROISOPROPANOL (HFIP)**

Haskell Laboratory Report No. 93-77

Medical Research Project No. 2296

Report by: *R. Culik*

**R. Culik
Senior Research Pathologist**

David P. Kelly

**D. P. Kelly
Toxicologist**

Approved for Pathology : *J. G. Aftosmis*

**J. G. Aftosmis
Manager, Pathology Section**

Approved for Inhalation Toxicology : *H. J. Trochimowicz*

**H. J. Trochimowicz
Chief, Inhalation Section**

RC:DPK:JGA:JHT:1jm

Date: March 3, 1977

Notebook No. 9159, pp. 59-81

EMBRYOTOKIC AND TERATOGENIC STUDIES IN RATS
WITH INHALED HEXAFLUOROISOPROPANOL (HFIP)

Haskell Laboratory Report No. 93-77

Medical Research Project No. 2296

This study was conducted by Mr. D. P. Kelly under the direction of Dr. H. J. Trochimowicz. Laparotomy, necropsy and gross pathology of the mothers and gross examination of the fetuses were carried out by Dr. R. Culik, Mrs. Jean A. Hostetler, Miss Alice V. Erwin, Mr. A. H. Stenholm, Mr. W. I. Swan and Mr. F. L. Ulmer. Clearing and Alizarin staining of the skeletons, and inspection of the fetuses were done by Dr. R. Culik, Miss Alice V. Erwin and Mr. A. H. Stenholm. Statistical valuation of the data was carried out by Mr. W. E. Fayerweather. The above work was supervised by Dr. J. G. Aftosmis.

EMBRYOTOXIC AND TERATOGENIC STUDIES IN RATS
WITH INHALED HEXAFLUOROISOPROPANOL (HFIP)

Haskell Laboratory Report No. 93-77

Medical Research Project No. 2296

SUMMARY AND CONCLUSIONS

1. Pregnant rats were exposed to hexafluoroisopropanol (HFIP) at concentrations of 30 and 200 ppm in air for six hours per day on day six through day 15 of gestation. The exposure had no effect on the body weight gains of the animals. No clinical signs of toxicity, changes in behavior or gross pathological changes in organs or tissues were observed in the treated animals.
2. The outcome of pregnancy, measured by the number of implantations, post-implantation resorptions and the live fetuses per litter, was not affected by the exposure. The inhaled material was not embryotoxic.
3. The exposure did not affect the embryonal development, measured by the weight and crown-rump length of the fetuses.
4. No major external, visceral or skeletal malformations were detected in fetuses exposed to the test material in utero. Minor anomalies and biological variants were related either to genetic background or chronologic age and not to the treatment. Under the conditions of this test HFIP was not teratogenic.

EMBRYOTOKIC AND TERATOGENIC STUDIES IN RATS
WITH INHALED HEXAFLUOROISOPROPANOL (HFIP)

Haskell Laboratory Report No. 93-77

Medical Research Project No. 2296

INTRODUCTION

Hexafluoroisopropanol (HFIP) is a chemical intermediate and effective solvent for polymers, dyes, polypeptides, inorganic salts and gases. As a part of a comprehensive toxicological evaluation of this material, embryotoxic and teratogenic potential was investigated in rats exposed to it by inhalation.

TEST MATERIAL

The test material was colorless liquid, 100% pure. It was submitted by E. F. Bauer of Central Research and Development Department and assigned Haskell No. 10,007.

PROCEDURE

The animals used were Charles River-CD albino female rats. They were bred at Charles River Breeding Laboratories*. Pregnancy was confirmed by finding sperm in the vaginal smear of females caged overnight with males. That morning was counted as day 1 of gestation. The animals were shipped in air-conditioned trucks directly to this laboratory the following day.

Seventy-five primigravida females, when received, were housed singly in suspended stainless steel wire cages and were assigned at random to three exposure groups**. On day six through day 15 of the gestation

* Charles River Breeding Laboratories, Inc., North Wilmington, Mass.

** One-half of the animals were bred on Day 0, the other half were bred on Day 0 + 1; all animals were received in one shipment, e.g., two and three days pregnant. The data are presented for the sum total as a unit.

period the groups of 25 rats were exposed in 1.4 m³ stainless steel chambers for six hours daily to 30 or 200 ppm (vol/vol in air) of HFIP. Concurrently, a group of 25 control animals was exposed to air in a similar chamber. After the daily exposure, the animals were kept in a holding area. Purina Laboratory Chow and water were available free choice one hour after the exposure until the next day. The food consumption was not measured. The animals were observed daily during and after exposure, and weighed twice weekly and on the day of laparotomy.

Vapors of hexafluoroisopropanol were produced by bubbling a stream of nitrogen through a glass vessel containing the liquid HFIP. The resulting vapor stream was fed into the air supply and into the exposure chambers. The concentrations of the test material in the chamber atmospheres were monitored every 15 minutes using gas chromatographic methods.

Prior to sacrifice, the females were anesthetized by chloroform inhalation. The uterus was exteriorized through a mid-line incision in the abdominal wall and was weighed. The uterine horns were opened and the fetuses removed. The following observations and enumerations were made.

- a. Number of corpora lutea in every ovary.
- b. Number of implantation sites in each horn.
- c. Number and location of live and dead fetuses.
- d. Number and location of late and early resorptions.
- e. Weight and crown-rump length of all live fetuses.
- f. Gross examination of all fetuses for external anomalies and malformations, from head to tail, under a long focal-length lens of 2½ magnification.

About one-half of the fetuses from each litter were preserved in 95% alcohol for subsequent maceration in 1% aqueous KOH, clearing and staining with Alizarin Red and examination to detect skeletal abnormalities. The remaining fetuses were fixed in Bouin's fluid for free-hand razor-blade sectioning (Wilson method) (1) and examination for visceral and neural anomalies under the dissecting microscope. The uterus and ovaries, from mothers in all groups, were examined for gross changes and preserved in Bouin's fluid for possible histopathologic examination. Other tissues and organs were examined grossly and discarded if found normal.

STATISTICAL EVALUATION

For statistical evaluation of the data, the litter was considered the experimental unit of treatment and observation. The Fisher exact probability test was used to evaluate the incidence of resorptions and abnormalities among the litters. Maternal and fetal body weights and body measurements were treated statistically by an analysis of variance and Least Significant Difference (LSD) test. The number of corpora lutea, implantations and live fetuses per litter were analyzed by the Mann-Whitney U-test. In all cases, the level of significance chosen was $p < 0.05$.

RESULTS

1. Analytical Exposure Levels

The average analytically-determined concentrations* of hexafluoroisopropanol during the 10-day exposure period were 29.4 ± 2.8 and 199.7 ± 17.5 ppm.

(1) Wilson, J. G. (1965). Methods for administering agents and detecting malformations in experimental animals. In: Teratology: Principles and Techniques. J. G. Wilson and J. Warkany, eds., Univ. of Chicago, pp. 262-277.

* Time-weighted average \pm standard deviation over the entire exposure period.

RESULTS (Continued)

2. Body Weight

Body weight gains of treated animals during and after the ten-day exposure period were not different from the control females. The initial and final mean body weight of the groups are indicated in Table I.

3. Clinical Observations

No clinical signs of toxicity or changes in behavior were observed in treated animals during and after the daily exposures.

4. Gross Changes at Necropsy

No gross pathological changes were observed in vital organs and tissues, including the ovaries and uterus, of exposed females. The ovaries and uterus were saved for possible microscopic examination.

5. Pregnancy Outcome and Fetal Development

The effect of inhaled HFIP on the outcome of pregnancy and fetal development is summarized in Table I. The exposures to concentrations of 30 and 200 ppm HFIP did not adversely affect the conception rate, the number of corpora lutea and implantations, the size of the litter and did not increase the incidence or alter the type of resorptions (early, late, total). The fetal body measurements in the test groups were slightly superior to those of the control group. The material did not interfere with the implantation of fertilized ova and did not affect the normal development of the fetus in utero.

6. Gross External, Soft Tissue and Skeletal Anomalies

The type and the incidence of fetal anomalies and variants among litters in the control and HFIP exposed groups are indicated in Table II. Examination of all fetuses at laparotomy for gross external abnormalities and of about one-half of the fetuses for skeletal and soft

RESULTS (Continued)

anomalies revealed no major malformations. Small subcutaneous hematomas and petechial hemorrhages on various parts of the fetus were evenly distributed in all groups. The incidence of hydro-nephrosis (dilatation of pelvis of the kidney) was significantly lower in groups exposed to HFIP compared with the control group. This defect occurs spontaneously in this strain of rats and is due to the genetic background. We find a comparable incidence of hydro-nephrosis, usually of one kidney, in adult control animals from various toxicity studies conducted at Haskell Laboratory.

Delayed ossification of sternabrae and hyoid and bipartite thoracic centra are related to chronological age of the fetuses and not to the exposure. Similarly we believe that the remnants of 14th rib(s) usually present as tiny spur(s), and the presence of wavy ribs in a few fetuses among the litters in almost every study, are of genetic origin and not treatment related.



HASKELL LABORATORY

cc: H. J. Trochimowicz
W. D. Krivanek
C. L. Dickinson
C. M. Barba

February 11, 1977

TO: R. Culik

STATISTICAL ANALYSIS OF RAT TERATOLOGIC STUDY
OF INHALED HEXAFLUOROISOPROPANOL (HFIP)

MR-2296 H-10,007

STATISTICAL REPORT NO. 5-77

Mean initial body weight of pregnant female rats exposed to 200 ppm HFIP was significantly higher than controls. No other significant differences were found.

Body weight and length measurements were analyzed by analysis of variance and least significant difference tests. The number of implantations, live fetuses, and resorptions per litter were analyzed with Wilcoxon's rank sum test. Counts of litters with certain attributes (such as litters with resorptions) were analyzed with Fisher's exact test. The litter was treated as the experimental unit and significance was set at the 0.05 probability level.

Report by:

W. E. Fayerweather
W. E. Fayerweather
Biostatistician

Approved by:

M. D. Krivanek
W. D. Krivanek
Chief, Physiology Section

WEF/jtd
Attachment

TABIE I

EFFECT OF RELATIVELY LOW DOSE OF HEPIC (HFIP) ON THE OUTCOME OF PREGNANCY AND FETAL DEVELOPMENT OF THE RAT

	Air Exposure Levels (ppm) of HFIP (1)		
	0	30	200
Females bred	25	25	25
Females pregnant (%)	20 (80.0)	20 (80.0)	18 (72.0)
Corpora lutea/pregnant female (2)	12.0 ± 2.1	12.6 ± 3.4	12.7 ± 2.0
Implantations/litter (2)	10.0 ± 3.4	9.1 ± 3.6	11.2 ± 2.1
Live fetuses/litter (2)	9.5 ± 3.2	8.6 ± 3.5	10.2 ± 1.9
Litters with early resorptions (%)	8 (40.0)	6 (30.0)	9 (50.0)
Litters with late resorptions (%)	0 (0.0)	0 (0.0)	0 (0.0)
Litters with dead fetuses (%)	0 (0.0)	0 (0.0)	1 (5.6)
Litters with partial resorptions (%)	8 (40.0)	6 (30.0)	9 (50.0)
Litters totally resorbed (%)	0 (0.0)	0 (0.0)	1 (5.6)
Resorptions/litter with resorptions (2)	1.5 ± 0.7	1.5 ± 0.4	2.1 ± 1.0
Initial body weight of pregnant female (gm) (2)	192.4 ± 13.8	195.5 ± 10.1	200.9 ± 9 (†)
Final body weight of pregnant female (gm) (2)	357.1 ± 33.6	355.7 ± 31.5	359.1 ± 33.6
Fetal weight (gm) (3)	3.66 ± 0.79	4.00 ± 0.96	3.85 ± 0.83
Fetal crown-rump length (cm) (3)	3.28 ± 0.27	3.42 ± 0.31	3.36 ± 0.30

(†) Significantly ($p < 0.05$) higher than the control group.

- (1) Administered by inhalation, 6 hours per day on days 6-15 of gestation; sacrificed on day 21.
- (2) Mean ± standard deviation.
- (3) Mean of litter means ± standard deviation.

**EFFECT OF INHALED HEXAFLUOROISOPROPANOL (HFIP)
ON THE INCIDENCE OF FETAL ANOMALIES IN RAT LITTERS**

	Air Exposure Levels (ppm) of HFIP (1)	
	0 (Control)	200
No. of litters (fetuses) examined	20 (189)	20 (172)
		18 (183)
	<u>Number of Litters Affected (No. of Fetuses)</u>	
<u>Gross</u>		
Small subcutaneous hematomas	4 (5)	7 (10)
Petechial hemorrhages	5 (10)	5 (6)
<u>Soft Tissue</u>		
Hydronephrosis	12 (22)	5 (7)↓
<u>Skeletal</u>		
Delayed ossification of 1 or more sternabrae	13 (44)	11 (38)
14th rud. rib(s) or spur(s)	16 (51)	20 (63)↑
Wavy ribs	5 (5)	2 (2)
Dipartite thoracic centra	3 (3)	7 (7)
Hyoid unossified	1 (2)	1 (3)
		8 (41)
		18 (58)
		1 (1)
		9 (18)↑
		1 (1)

(1) Administered by inhalation, 6 hours daily on days 6-15 of gestation; sacrificed on day 21.

(↑) Significantly ($p < 0.05$) higher, lower than the control group by Fisher's Exact Probability Test.

CERTIFICATE OF AUTHENTICITY

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