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SOLVENT REFINED COAL (SRC) PROCESS: HEALTH PROGRAMS

Final Report of Subcontract No. 10 for June 1, 1976—June 9, 1978

November 1981

Work Performed Under Contract No. AC05-76ET10104

Gulf Science and Technology Company
Toxicology Department
Pittsburgh, Pennsylvania

and

The Pittsburg & Midway Coal Mining Company
Englewood, Colorado

U. S. DEPARTMENT OF ENERGY

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SOLVENT REFINED COAL (SRC) PROCESS: HEALTH PROGRAMS

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INTERIM REPORT NO. 39
VOLUME III: PILOT PLANT DEVELOPMENT WORK
PART 4: INDUSTRIAL HYGIENE, CLINICAL AND TOXICOLOGICAL PROGRAMS
FINAL REPORT OF SUBCONTRACT NO. 10
FOR THE PERIOD
JUNE 1, 1976 THROUGH JUNE 9, 1978

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November, 1981

PREPARED FOR THE
U. S. DEPARTMENT OF ENERGY
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ABSTRACT

This report summarizes the toxicological studies on SRC-I materials completed under Subcontract No. 10 as part of the Health Programs under the Solvent Refined Coal (SRC) Process Contract during the total period of the subcontract, June 1, 1976 through June 9, 1978. The studies were conducted by Industrial Bio-Test Laboratories (IBT) as the subcontractor. A number of acute studies were completed on the products and intermediate streams as well as several subchronic studies. In addition, preliminary dose-ranging, or pilot, studies were completed. None of the materials exhibited high toxicities when administered orally, dermally, or by the inhalation route. Three of the materials proved to be severely or extremely irritating to the eyes. The pilot dermal and teratogenesis studies revealed some evidence of decreased viability in offspring and reduced fetal body weights. The subcontract was terminated for convenience on June 9, 1978 when it became apparent that IBT could not satisfactorily continue the studies.

I. SUMMARY

Since the start-up of the Solvent Refined Coal Pilot Plant at Fort Lewis, Washington, in 1974, a very comprehensive health program has been in effect. Three objectives of the program are:

1. Protection of the worker from exposure to materials which could potentially result in adverse health effects through repeated and prolonged exposures.
2. Monitoring of the worker atmosphere to measure the extent and nature of exposure both from a health standpoint and to identify specific areas of the process for additional engineering controls to minimize or eliminate harmful emissions and for design considerations in future larger scale plants.
3. An assessment of the toxic hazards of the materials through extensive animal bioassay studies.

The four major efforts in the program are an industrial hygiene monitoring program, a clinical medical examination program, a personal hygiene and educational program, and a toxicology program. This report focuses on the toxicology program and specifically, on the initial studies completed on SRC-I materials.

A program for the toxicological evaluation of various materials associated with the SRC-I Solvent Refined Coal process was developed and a subcontract for the performance of the studies was awarded to Industrial Bio-Test Laboratories late in 1976. The principal objective of the program was to evaluate potential health hazards to plant personnel, transportors and users of SRC materials. Some portions of the program were carried out during 1977 but, unfortunately, the contracting laboratory encountered increasingly severe criticism from government regulatory agencies for deficiencies in their past work for other sponsors. This culminated, early in 1978, in the laboratory stopping all programs and placing itself on the market for sale. Thus, only a part of the planned program on SRC-I materials was completed. The subcontract was subsequently terminated for convenience on June 9, 1978.

There were three major components to this SRC-I program: (1) a series of acute tests designed to provide some insight into the short-term effects of single exposures such as in spills and accidents and also to provide guidance for dose level selection for longer term tests, (2) intermediate length (subchronic) tests designed to evaluate the teratogenic and other latent toxic effects of multiple exposures, and (3) long-term (chronic) tests designed to evaluate the toxic and carcinogenic effects of long-term exposure to these materials. The testing program employed the conventional laboratory species (e.g. rats, mice, rabbits) and emphasized the dermal and inhalation routes of exposure. Of the various acute, subchronic, and chronic tests initially planned in the program, only the following studies were completed:

- o Acute oral toxicity - dry mineral residue, SRC product
- o Acute dermal toxicity - dry mineral residue, SRC product
- o Acute vapor toxicity - process solvent, light oil, wash solvent, coal slurry, filter feed, wet mineral residue
- o Acute aerosol toxicity - process solvent, wash solvent
- o Acute eye irritation - Process solvent, coal slurry, filter feed, dry mineral residue, wet mineral residue, light oil, wash solvent, SRC product
- o Subchronic vapor toxicity - Process solvent, coal slurry, filter feed, wet mineral residue, light oil, wash solvent
- o Pilot dermal teratogenicity - Process solvent, filter feed, wet mineral residue, light oil, wash solvent, SRC product
- o Pilot skin painting - Process solvent, coal slurry, filter feed, wet mineral residue, light oil, wash solvent

The results obtained in this program on SRC-I materials may be summarized as follows:

1. Dry mineral residue and the SRC product have a low order of toxicity when administered orally or dermally.
2. None of the six volatile materials exhibited a high toxicity when vapors were administered by the inhalation route.
3. Three of the materials, wet mineral residue, light oil, and wash solvent, proved to be severely or extremely irritating to the eyes when examined in the rabbit eye irritation test. The damage from two of these, wet mineral residue and wash solvent, did not appreciably diminish in 14 days.
4. An early chance observation of corneal opacity in mice in a dose-ranging skin painting study could not be verified in a carefully controlled subsequent study.
5. Pilot dermal and inhalation teratogenesis studies revealed some evidence of decreased viability in offspring and reduced fetal body weight in rabbits. Verification of these observations must await the performance of the full-scale, definitive teratogenesis studies.

Also, in line with current requirements and guidelines governing such toxicological investigations, careful auditing of the research reports

embodying the findings was conducted in order to validate the reported observations and conclusions.

II. INTRODUCTION

For several decades, research has been conducted in the United States to find improved techniques to liquefy coal with the dual objectives of reducing costs and utilizing our vast domestic reserves of coal. Most of this research has been conducted by industry and sponsored by various government agencies. One such program initiated in 1962 was the development of an extraction process to deash coal. This process is now known as the Solvent Refined Coal (SRC) process. Initial development of the process was by Spencer Chemical Company, now a part of Gulf Oil Corporation, under the sponsorship of the Office of Coal Research (OCR). By 1965, its technical feasibility was proven and in 1966, OCR contracted with The Pittsburg & Midway Coal Mining Co. (P&M), subsidiary of Gulf Oil, to continue the development through construction of a pilot plant with a capacity of 50 tons of coal per day. The design was completed in 1969, construction started at a site in Ft. Lewis, Washington in 1972, and the plant was first operated in September, 1974. The plant was designed to produce a fuel that is solid at ambient temperature and can be burned like pulverized coal. This mode of operation has since become known as the SRC-I process.

A part of this pilot plant development work, conducted under contract EX-76-C-01-0496 with the U. S. Department of Energy, was a program for the toxicological evaluation of various materials associated with the process. The principal objective of such evaluation was to better define potential health hazards to plant personnel, transporters, and users of SRC materials. Toward this end, a toxicology program was developed and a subcontract for the performance of the program was awarded to Industrial Bio-Test Laboratories (IBT) late in 1976. Some portions of the program were carried out during 1977 but, unfortunately, during this period IBT encountered increasingly severe criticism from government regulatory agencies for deficiencies in previous work for other sponsors. This criticism led P&M to terminate the IBT subcontract in 1978, and culminated in the dissolution of IBT as a toxicology testing laboratory.

During the period from initial plant operation in 1974 to approximately the time of the IBT subcontract cancellation in 1978, the pilot plant operated with success in the SRC-I mode. The most frequently recurring problem was with filtration. One very promising variation of the SRC-I process did not involve filtration. Without the complexities of filtration, this modified mode, now known as the SRC-II process, was quickly developed. The occasion of the development of this new process was utilized to re-evaluate the toxicology program. The result was the design of a new program which not only provided for continuing the evaluation of SRC-I materials, but also included an evaluation of new materials associated with the SRC-II process. This new program is presently underway, but is not the

subject of this report. Instead, the purpose of this report is to present the results of toxicology testing conducted on SRC-I materials by Industrial Bio-Test Laboratories, Inc. Included in the report are tabulations of the studies performed, summarization of the program findings, results of quality assurance reviews of these studies and the individual study reports submitted by IBT.

III. SCOPE OF PROGRAM

There were three major components in the original SRC-I program: (1) a series of acute studies designed to provide some insight into the short-term effects of single exposures such as in the cases of spills and accidents, and also to provide guidance for dose level selection for longer term studies, (2) intermediate length (subchronic) studies designed to evaluate the teratogenic and other latent toxic effects of multiple exposures, and (3) long-term (chronic) studies designed to evaluate the toxic and carcinogenic effects of long-term exposure to these materials. The testing program employed the conventional laboratory species (e.g. rats, mice, rabbits) and emphasized the dermal and inhalation routes of exposure.

Five types of acute studies, four types of subchronic studies, and two types of chronic studies were planned. The title, purpose, and brief descriptions of the various studies are presented below:

A. Acute Toxicity Studies:

Acute Oral Toxicity (LD50) Study in Rats - This procedure is designed to provide an estimate of the dose lethal to 50% of the test animals when administered orally. This is a rapid test that provides an approximate estimate of acute toxicity. Groups of unfasted rats receive graded doses of the test materials suitably diluted and administered by stomach tube. The incidence of death is recorded over a 14-day observation period, and the LD50 is estimated by the moving average technique.

Acute Dermal Toxicity (LD50) Study in Rabbits - This procedure is designed as an appraisal of the acute dermal toxicity of a test material. Graded doses of the material are applied to unabraded and abraded clipped skin of groups of rabbits and maintained in contact for 24 hours. All rabbits are observed for mortality and toxic effect on the day of application and daily thereafter for 14 days. Necropsies are performed on any animal that dies and of all animals at the end of 14 days. The acute dermal LD50 is calculated.

Guinea Pig Sensitization Study - Albino guinea pigs are used to evaluate the skin sensitizing properties of each test material. Three sensitization induction applications are applied each week, on alternate days, for a total of three weeks. Local reactions are observed at 24 and 48 hours. The challenge dose is administered two weeks after

the last sensitizing application. Reactions among the test animals following the challenge dose are observed to determine if the material is sensitizing.

Eye Irritation Study in Rabbits - This study determines if a test material causes permanent eye damage or serious temporary damage. The standard test as described by J. H. Draize is utilized. Standard amounts of the test material are instilled into one eye of each animal; the other eye serves as the control. Observations for eye irritation are made at one, two, three, four and seven days, or as long as the injury persists, for a period of two weeks. Results are reported by the standard Draize scoring technique.

Acute Inhalation Toxicity (LC50) Study in Rats - This procedure is designed to provide an estimate of the four-hour LC50: the concentration lethal to 50% of the animals for four-hour exposure to materials in a vapor or aerosol form. Groups of male rats are exposed for four hours to graded airborne concentrations of the test material. The incidence of death is recorded over a 14-day observation period. The four-hour inhalation LC50 is calculated.

B. Subchronic Toxicity Studies:

Subchronic Dermal Toxicity Study in Rabbits - This test is designed to provide an evaluation of possible latent effects following prolonged skin contact with a test material. Four groups of rabbits are used: one group, the control, receives a placebo; the other three groups receive a low, intermediate or high dose level of the test material. A total of 65 applications, five per week for 13 weeks, are administered. A subset of the animals is sacrificed and necropsied after the fifteenth application. The remaining animals are sacrificed and necropsied 72 hours after the last application. Clinical pathology studies are performed on all animals prior to treatment, at the end of the three-week (15 application) phase, and on all remaining animals at termination. All animals are necropsied and selected tissues fixed and examined microscopically.

Subchronic Inhalation Toxicity Study in Mice - This study was added to the series after corneal opacity was observed in many animals, including controls, in a pilot dermal study. It was unclear if the effect was inherent in the group of test animals or if it was caused by some vapor phase transfer from the test materials. Test and control animals were exposed in inhalation chambers to selected concentrations of test materials six hours per day, five days per week, for four weeks. Observations of mortality and toxicity were made daily. The eyes of animals were closely examined for lesions.

Dermal Teratology Studies in Rats and Rabbits - This study is designed to detect possible teratogenic effects of a test material in albino rats

and rabbits. Test materials are applied to the shaved backs of the animals at one control and two test levels on the sixth through the fifteenth day of gestation for rats; and the sixth through the eighteenth day of gestation for rabbits. Body weights, mortality, and reactions are noted throughout the investigation. All female rats are sacrificed on the twentieth day of gestation; all rabbits on the twenty-ninth day. Fetuses are examined for their number, viability, weights, and abnormalities. Dams are examined for fetal swellings, implantation sites, resorption sites, and any uterine abnormalities. Fetus skeletal development is evaluated as well as internal organ development.

Inhalation Teratology Study in Rats and Rabbits - This study is designed to detect possible teratogenic effects of a test material in albino rats and albino rabbits when exposure is by the inhalation route. The test materials are administered by inhalation exposure for six hours each day in specially constructed inhalation chambers. Study duration, record maintenance and experiment evaluation closely parallels that of the dermal teratology study.

C. Chronic Toxicity/Carcinogenicity Study:

Two-Year Skin Painting Study in Mice - This is an investigation of possible carcinogenic effects from dermal exposure to a test material. A control group and two dose levels of the test material are studied. The test or control material is applied to the shaved back of the animals twice weekly. Individual body weights are recorded at week 0, at weekly intervals for the next 13 weeks, and at monthly intervals thereafter. Daily observations are made for signs of toxicity and mortality. Necropsies are performed on all surviving animals at the end of two years. Animals that die during the study and moribund animals that are sacrificed are also necropsied. The incidence, size and disposition of tumors are recorded in detail. Microscopic pathology of selected tissues and gross lesions is performed.

Two-Year Inhalation Carcinogenicity Study in Rats - This study is designed to detect possible carcinogenic effects from inhalation of an airborne form of a test material. Animals are placed in one of three groups: a control group exposed to uncontaminated air, a low-level group for each test material, and a high-level group for each test material. The animals are exposed six hours per day, five days per week, for 104 weeks. Appropriate analytical monitoring of air samples is performed to assure that desired chamber concentrations are maintained. Body weights are recorded at week 0, at weekly intervals for the next 13 weeks, and at monthly intervals thereafter. Observations are made daily for mortality and weekly for evidence of toxicity. Detailed records are maintained of the incidence, size, identification, and disposition of tumors as the study progresses. Necropsies are performed on moribund animals and on the survivors at study termina-

tion. Appropriate tissues are taken and examined. Special attention is directed to the examination of the respiratory tract. Nine process-related materials were selected for partial or complete evaluation in this program. Selection of materials was based primarily on the potential for human exposure through contact in the workplace, in transport, or in commercial applications with emphasis on studying materials that were unique to the process. The slate of selected materials ranged, in terms of the process, from unreacted pulverized coal to the solid product fuel. Intermediate process streams included the coal slurry feed to the dissolver (Coal slurry), the product slurry from the dissolver following raw gas removal (filter feed), wet and dry residues from the filtration of the product slurry (wet and dry mineral residue), and liquids recovered from the filtered product slurry (light oil, wash solvent and process solvent).

The individual studies included for each test material in the program are represented in Table 1. This table also identifies those studies which were conducted by Industrial Bio-Test Laboratories before the contract was terminated. Of the 40 acute, 35 subchronic, and 11 chronic studies planned, 20 acute and 6 subchronic studies were completed. In addition, preliminary dose-range, or pilot, studies were conducted for 16 of the remaining subchronic studies and 6 of the chronic studies.

TABLE 1
Original SRC Toxicology Program -
SRC-I Materials

Test \ Material	Process Solvent	Coal Slurry	Filter Feed	Dry Mineral Residue	Wet Mineral Residue	Light Oil	Wash Solvent	Pulverized SRC Product	Pulverized Coal
Acute Oral LD50 in Rats	X	X	X	C	X	X	X	C	
Acute Dermal LD50 in Rabbits	X	X	X	C	X	X	X	C	
Guinea Pig Skin Sensitization	X	X	X	X	X	X	X	X	
Eye Irritation in Rabbits	C	C	C	C	C	C	C	C	
Acute Inhalation LC50 in Rats: Vapor Aerosol	C C	C	C		C	C	C C		
Subchronic Dermal Toxicity in Rabbits	X						X	X	
Subchronic Inhalation Toxicity in Mice	C	C	C		C	C	C		
Dermal Teratogenicity in Rats and Rabbits	X P		X P		X P	X P	X P	X P	
Inhalation Teratogenicity in Rats and Rabbits	P P		D D	P P		X X	P P	P P	P P
Two-year Skin Painting in Mice	P	P	P		P	P	P	X	
Two-year Inhalation Carcinogenesis in Rats	X			X			X	X	

X = material to be studied.

C = study completed by Industrial Bio-Test Laboratories

P = pilot study completed

D = study deleted, impractical to aerosolize filter feed

IV. RESULTS AND DISCUSSION

As shown in Table 1, the following acute studies were completed:

- o Acute oral toxicity - dry mineral residue, SRC product
- o Acute dermal toxicity - dry mineral residue, SRC product
- o Acute vapor toxicity - process solvent, light oil, wash solvent, coal slurry, filter feed, wet mineral residue
- o Acute aerosol toxicity - process solvent, wash solvent
- o Acute eye irritation - process solvent, coal slurry, filter feed, dry mineral residue, wet mineral residue, light oil, wash solvent, SRC product

It was found that the toxicities of dry mineral residue and SRC product were so low that the results could only be expressed in the form:

Oral LD50 > 15,380 mg/kg
Dermal LD50 > 10,250 mg/kg

No instances of high toxicity were noted in the acute vapor inhalation studies, as the following results show:

Process solvent	LC50 > 1.60 m/l
Coal Slurry	LC50 > 0.44 mg/l
Filter feed	LC50 > 1.14 mg/l
Wet mineral residue	LC50 > 3.94 mg/l
Light Oil	LC50 > 71.6 mg/l
Wash solvent	LC50 > 7.91 mg/l

When process solvent and wash solvent were administered as aerosols in acute inhalation studies, the results observed were:

Process solvent	LC50 > 7.6 mg/l
Wash solvent	LC50 = 16.7 mg/l

The results of the acute eye irritation studies were of considerable interest. The standard Draize technique in rabbits was employed, and the scores obtained and classification of the findings were as follows:

	<u>Maximum Score</u>	<u>Irritancy Classification</u>
Process Solvent	23.8/110.0	Mild
Coal Slurry	28.1	Moderate
Filter Feed	32.1	Moderate
Dry mineral residue	6.0	Minimal
Wet mineral residue	73.6	Extreme
Light Oil	40.6	Severe
Wash solvent	49.3	Severe
SRC product	7.7	Minimal

Three materials, wet mineral residue, light oil and wash solvent, proved to be severely or extremely irritating to the rabbit eye. It was noted that the extent of injury, as estimated by the Draize score, was essentially unchanged after 14 days in the cases of wet mineral residue and wash solvent, but there was appreciable resolution after 14 days in the case of light oil. These findings were considered to constitute a reportable result under the provisions of the Toxic Substances Control Act, Section 8(e), and they were duly communicated to the Environmental Protection Agency. Phenolic compounds present in these materials are thought to be the active agents in the observed eye irritation.

While the results given above constitute the definitive findings from the SRC-I program before it was terminated, some additional observations were made in preparatory dose-ranging, or pilot studies. A dose-ranging study was carried out in which small groups of mice were skin painted twice weekly for 30 days with one of the materials. All materials, with the exception of dry mineral residue, were studied. Satisfactory dose levels for use in the planned two-year skin painting studies were established, but an unusual observation was made at termination of the 30 day trial. Some mice in each of the test groups and in the control group exhibited corneal opacity. Since the effect was seen in control animals as well as those treated with the various SRC materials, one hypothesis was that a volatile constituent from one or more of the materials may have contaminated the general laboratory room air. Therefore, a special definitive study was designed and conducted in which mice were exposed for 30 days to the vapors of the volatile constituents of each material. The studies were conducted in isolated chambers so that the possibility of cross-contamination was eliminated and daily observations of the eyes were performed. No effects were seen in the eyes of the mice in any group, including controls, in this carefully controlled experiment. It was concluded, therefore, that the original observation was artifactual in nature and not associated with exposure to the SRC materials.

Pilot teratogenesis studies were conducted with dry mineral residue, the SRC product, and also on powdered coal, with these solids being administered by inhalation in the form of aerosols. Mean rabbit fetal body weights were found to be lower in the SRC product groups than in the controls and this effect was also detected in coal-exposed rabbits, but only at the intermediate dose level. No effects were seen in similarly treated rats. Again, with the small number of animals employed, these superficially apparent findings will require verification in final, full-scale investigations.

The results for SRC-I materials studies in this program may be summarized as follows:

- (1) Dry mineral residue and the SRC product have a low order of toxicity when administered orally or dermally.
- (2) None of the six volatile materials exhibited a high toxicity when vapors were administered by the inhalation route.
- (3) Three of the materials, wet mineral residue, light oil, and wash solvent, proved to be severely or extremely irritating to the eyes when examined in the rabbit eye irritation test. The damage from two of these, wet mineral residue and wash solvent, did not appreciably diminish in 14 days.
- (4) An early chance observation of corneal opacity in mice in a dose-ranging skin painting study could not be verified in a carefully controlled subsequent study.
- (5) Pilot dermal and inhalation teratogenesis studies revealed some evidence of decreased viability in offspring and reduced fetal body weights in rabbits. Verification of these observations must await the performance of the full-scale, definitive teratogenesis studies.

Finally, in keeping with modern requirements governing toxicological investigations of the types described above, careful auditing of the research reports embodying the findings was conducted in order to validate the reported observations and conclusions. The results of these audits are presented in the next section of this report.

V. Quality Assurance Review

A. Description

Concern first expressed by the U. S. Environmental Protection Agency (EPA) and the U. S. Food and Drug Administration (FDA) in the summer of 1977, regarding the validity of toxicological tests conducted by IBT, prompted P&M to initiate comprehensive audits of the studies performed by IBT as a part of this program. Although audits of these particular

studies were not required by the Federal Agencies, audits of these same types of IBT studies were required of pesticide registrants by EPA's Office of Pesticide Programs (OPP), and of food product applicants by the FDA. It was felt that the agencies' positions on these matters raised sufficient question of all IBT work to necessitate audits of the SRC studies.

The SRC study audits were conducted by a specialized group of Quality Assurance Toxicologists within the Gulf Science and Technology Company, and were modeled after recommended procedures described by the EPA's OPP in a letter dated July 27, 1977, and in a subsequent EPA document entitled: "Guide to Pesticide Registrants for Audits of IBT Studies". Based upon these guidelines, the Gulf Quality Assurance Unit (QAU) developed a questionnaire that addressed questions about laboratory capabilities and standard laboratory practices and procedures. Additional questionnaires were developed that addressed specific questions about study conduct and report accuracy. These questionnaires served as the basic framework for the audits. Copies of the questionnaires are presented in Appendices A, B, and C.

During the course of auditing the 48 studies performed by IBT as a part of this program, six site visits were made to IBT facilities by members of the QAU, and more than five hundred man-hours were logged. Audits routinely involved evaluation of:

- a. laboratory facilities, capabilities, and standard operating procedures as determined by a walk-through inspection and questioning of laboratory personnel.
- b. qualifications of laboratory personnel based upon their curricula vitae.
- c. the differences, if any, between the intended study design (if defined in a study protocol), and the actual study design as reflected in the laboratory records maintained.
- d. the accuracy and completeness of the reported data as compared to the laboratory records maintained.

A typical auditing procedure involved completion of the questionnaires presented in the Appendices, based upon information gained by the four types of evaluations listed above. Specific questions that remained unanswered following these evaluations were forwarded in writing to IBT management for their response. When the written response was obtained, it was incorporated into the data pool for the affected studies.

Based upon the audit findings, the validity of each of the 48 studies was assessed. Obviously, in this type of after-the-fact audit, little evidence is gained regarding the accuracy of the recorded data. On the other hand, much is learned about the ability of IBT to follow an intended experimental design and to accurately report recorded information. Therefore, the

individual validation statements prepared by the QAU primarily addressed the report accuracy, and addressed secondarily the precision or scientific acceptability of the laboratory procedures employed.

B. Findings

Audits completed following receipt of final study reports from IBT, and validation statements prepared by the QAU based on those audits, reveal that 38 of the 48 studies performed by IBT were useful for their intended purposes, and final reports of these studies were found to accurately reflect the laboratory records of study results.

The final reports of the 10 pilot inhalation teratology studies were found to be unacceptable, due to the number and magnitude of errors contained in the reports when compared to laboratory records of study results. In spite of the poor quality of the reports submitted for these 10 studies, the results achieved in these tests, which were pilot studies intended primarily to define dosage concentrations for use in definitive studies, are considered to have adequately answered the questions which gave rise to the studies.

A complete listing of the 48 study titles and the QAU decisions regarding report acceptability is presented in Table 2.

TABLE 2

IBT Toxicology Studies of SRC I Materials -
Individual Study Findings of the Gulf Quality Assurance Unit

Acute Studies	Study	Final Report
		Acceptable
	Acute Oral Toxicity Study of Dry Mineral Residue in Rats	Acceptable
	Acute Oral Toxicity Study of SRC Product in Rats	Acceptable
	Acute Dermal Toxicity Study of Dry Mineral Residue in Rabbits	Acceptable
	Acute Dermal Toxicity Study of SRC Product in Rabbits	Acceptable
	Acute Vapor Inhalation Toxicity Study of Process Solvent in Rats	Acceptable
	Acute Vapor Inhalation Toxicity Study of Coal Slurry in Mice	Acceptable
	Acute Heated Vapor Inhalation Toxicity Study of Filter Feed in Mice	Acceptable
	Acute Vapor Inhalation Toxicity Study of Wet Mineral Residue in Mice	Acceptable
	Acute Vapor Inhalation Toxicity Study of Light Oil in Rats	Acceptable
	Acute Vapor Inhalation Toxicity Study of Wash Solvent in Rats	Acceptable
	Acute Aerosol Inhalation Toxicity Study of Process Solvent in Rats	Acceptable
	Acute Aerosol Inhalation Toxicity Study of Wash Solvent in Rats	Acceptable
	Acute Eye Irritation Study of Process Solvent in Rabbits	Acceptable
	Acute Eye Irritation Study of Coal Slurry in Rabbits	Acceptable
	Acute Eye Irritation Study of Filter Feed in Rabbits	Acceptable
	Acute Eye Irritation Study of Dry Mineral Residue in Rabbits	Acceptable
	Acute Eye Irritation Study of Wet Mineral Residue in Rabbits	Acceptable
	Acute Eye Irritation Study of Light Oil in Rabbits	Acceptable

TABLE 2 (cont.)

	<u>Study</u>	<u>Final Report</u>
Acute Studies (cont.)	Acute Eye Irritation Study of Wash Solvent in Rabbits	Acceptable
	Acute Eye Irritation Study of SRC Product in Rabbits	Acceptable
Subchronic Studies	Four-Week Vapor Inhalation Toxicity Study of Process Solvent in Mice	Acceptable
	Four-Week Vapor Inhalation Toxicity Study of Coal Slurry in Mice	Acceptable
	Four-Week Heated Vapor Inhalation Toxicity Study of Filter Feed in Mice	Acceptable
	Four-Week Vapor Inhalation Toxicity Study of Wet Mineral Residue in Mice	Acceptable
	Four-Week Vapor Inhalation Toxicity Study of Light Oil in Mice	Acceptable
	Four-Week Vapor Inhalation Toxicity Study of Wash Solvent in Mice	Acceptable
Pilot Studies	33-Day Pilot Skin Painting Study of Process Solvent in Mice	Acceptable
	33-Day Pilot Skin Painting Study of Coal Slurry in Mice	Acceptable
	33-Day Pilot Skin Painting Study of Filter Feed in Mice	Acceptable
	33-Day Pilot Skin Painting Study of Wet Mineral Residue in Mice	Acceptable
	33-Day Pilot Skin Painting Study of Light Oil in Mice	Acceptable

TABLE 2 (cont.)

Pilot Studies (cont.)	Study	Final Report
		Acceptable
	33-Day Pilot Skin Painting Study of Wash Solvent in Mice	Acceptable
	Pilot Dermal Teratology Study of Process Solvent in Rabbits	Acceptable
	Pilot Dermal Teratology Study of Filter Feed in Rabbits	Acceptable
	Pilot Dermal Teratology Study of Wet Mineral Residue in Rabbits	Acceptable
	Pilot Dermal Teratology Study of Light Oil in Rabbits	Acceptable
	Pilot Dermal Teratology Study of Wash Solvent in Rabbits	Acceptable
	Pilot Dermal Teratology Study of SRC Product in Rabbits	Acceptable
	Pilot Inhalation Teratology Study of Process Solvent in Rats	Unacceptable
	Pilot Inhalation Teratology Study of Process Solvent in Rabbits	Unacceptable
	Pilot Inhalation Teratology Study of Wash Solvent in Rats	Unacceptable
	Pilot Inhalation Teratology Study of Wash Solvent in Rabbits	Unacceptable
	Pilot Inhalation Teratology Study of Dry Mineral Residue in Rats	Unacceptable
	Pilot Inhalation Teratology Study of Dry Mineral Residue in Rabbits	Unacceptable
	Pilot Inhalation Teratology Study of SRC Product in Rats	Unacceptable
	Pilot Inhalation Teratology Study of SRC Product in Rabbits	Unacceptable
	Pilot Inhalation Teratology Study of Pulverized Coal in Rats	Unacceptable
	Pilot Inhalation Teratology Study of Pulverized Coal in Rabbits	Unacceptable

APPENDIX A
NONCLINICAL LABORATORY
INSPECTION REPORT FORMS

GULF TOXICOLOGY DEPARTMENT

NONCLINICAL LABORATORY INSPECTION REPORT

Laboratory _____

Date of Inspection _____

Inspected by _____

Type of Laboratory: ☐ Sponsor
☐ Contract
☐ University
☐ Other (explain) _____

NONCLINICAL LABORATORY INSPECTION REPORT

A. TESTING FACILITIES

ITEM	YES	NO	N/A	NARRATIVE
1. The testing facility in general is of suitable size, adequate construction and properly located to perform nonclinical laboratory studies. Defined and, if necessary, separate areas are provided.				
2. Adequate space is provided for administration, supervision, and direction of the testing facility as well as satisfactory facilities for toilets, lockers, showers with hot and cold water, and air driers or single use towels, plus all necessary accouterments in accordance with regulations set forth by the OSHA in 29 CFR.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "TESTING FACILITIES"
IN THE NARRATIVE PORTION OF THE REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

B. PERSONNEL

	YES	NO	N/A	NARRATIVE
1. Obtain organizational chart and list of personnel.				
2. Employees practice good sanitation and health habits.				
3. Employees follow standard operating procedures for health and safety and have adequate laboratory clothing appropriate for their duties and to prevent microbiological or chemical contamination of the test substance.				
4. All employees are instructed to report to supervisory personnel any and all health or medical conditions that may be considered to adversely affect the study.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "PERSONNEL"
IN THE NARRATIVE PORTION OF THE REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

C. QUALITY ASSURANCE UNIT

ITEM	YES	NO	N/A	NARRATIVE
1. There is a quality assurance unit (QAU).				
2. A master schedule sheet of all nonclinical laboratory studies is maintained by the QAU.				
3. Copies of all protocols and standard operating procedures are maintained by the QAU.				
4. Critical reviews of final reports are made to assure accuracy of description with respect to methods; and,				
5. Standard operating procedures; and,				
6. Observations; and				
7. Raw data; and,				
8. Results (assuring that all adverse findings are indeed included in the final report).				
9. Procedures are written that describe the responsibilities of the QAU and the records it maintains.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING
"QUALITY ASSURANCE UNIT" IN THE NARRATIVE PORTION OF THE REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

D. EQUIPMENT

ITEM	YES	NO	N/A	NARRATIVE
1. Equipment of appropriate design and adequate capacity is available to obtain values reported.				
2. Location of equipment permits easy operation, cleaning and maintenance; and,				
3. Is cleaned, inspected and maintained regularly.				
4. There are written standard operating procedures which describe in detail the methods, materials and schedules to be used in the routine inspection, cleaning, maintenance, testing and calibration of equipment; and,				
5. The specific remedial actions to be taken in the event of failure or malfunction of equipment; and,				
6. Designates the individual responsible for each of the operations.				
7. Copies of the standard operating procedures are available to laboratory personnel.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "EQUIPMENT"
IN THE NARRATIVE PORTION OF THE REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

E. TESTING FACILITY OPERATION

ITEM	YES	NO	N/A	NARRATIVE
1. Separate laboratory space is provided for the performance of routine procedures or categories of procedures; and,				
2. Separate laboratory space is provided for the performance of specialized activities such as aseptic surgery, intensive care, necropsy and radiography.				
3. Spaces for cleaning, sterilizing, and maintaining equipment and supplies used during the course of the study are separate from the areas housing the test systems.				
4. Studies involving radioactive or other biohazardous materials are carried out in special facilities or areas which provide protection to personnel, test systems, and the external environment against contamination or unnecessary radiation exposure or infection.				
5. Persons possessing and using radioactive materials are licensed in accordance with the Nuclear Regulatory Commission regulations or meet the requirements of an agreement state.				
6. Special procedures are employed for the handling of other biohazardous materials.				
7. Written standard operating procedures (which at least meet GLP requirements) are maintained detailing the methods to be used in performing nonclinical laboratory studies.				
8. Standard operating procedures are established for animal room preparation; and,				
9. Animal care; and,				
10. Test and control substances: receipt, identification, strength, quality, purity, stability, storage, handling, mixing, sampling and administration; and,				
11. Test system observations; and,				
12. Laboratory tests; and,				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "TESTING FACILITY OPERATION" IN THE NARRATIVE PORTION OF THE REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

E. TESTING FACILITY OPERATION (continued)

ITEM	YES	NO	N/A	NARRATIVE
13. Handling of animals found moribund or dead during study; and				
14. Necropsy of animals or post-mortem examination of animals; and,				
15. Preparation of specimens; and,				
16. Histopathology; and,				
17. Data handling, storage, and retrieval; and				
18. Preparation and validation of final study report.				
19. A historical file of standard operating procedures annotating effective dates and dates of revisions is maintained.				
20. The relevant standard operating procedures are available at all times in the immediate bench area of personnel performing the procedures.				
21. All reagents and solutions in the laboratory areas are labeled adequately.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "TESTING FACILITY OPERATION"
IN THE NARRATIVE PORTION OF THE REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

F. ANIMAL CARE

ITEM	YES	NO	N/A	NARRATIVE
1. The testing facilities which utilize cats, dogs, guinea pigs, hamsters, rabbits, or nonhuman primates have been inspected by the U.S. Dept. of Agriculture Animals, Plant, Health Inspection Service, and found to be in compliance with the Animal Welfare Act of 1970 (9 CFR Part 3) within the past two years. Indicate date and results; and/or				
2. Feed and water used for animals are analyzed periodically for the presence of known interfering contaminants.				
3. A program for adequate veterinary care and humane treatment has been established and is supervised by a doctor of veterinary medicine (indicate name of DVM) for studies involving cats, dogs, guinea pigs, hamsters; rabbits, or nonhuman primates; and,				
4. For studies involving other animals by either a doctor of veterinary medicine or by other qualified persons (indicate name and qualifications).				
5. Animals either known to be, or suspected of being diseased, or carriers of a disease, are isolated in an area contiguous with or near the animal housing area.				
6. Animals are free of any naturally occurring diseases or conditions that might interfere with the purpose or conduct of the study.				
7. The diagnosis, authorization for and description of the treatment (including dates of treatment of animals involved) of test systems is adequately documented.				
8. Methods for the unique and permanent identification of all animals when needed have been developed and applied to preclude mix-up of animals and/or their tissues; and,				
9. Routine or specialized housing of animals of different species, or of the same species used for different studies, is adequate to preclude interspecies transmission of infection, mix-up, or other events that may affect the outcome of a study or studies.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "ANIMAL CARE"
IN THE NARRATIVE PORTION OF THE REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

F. ANIMAL CARE (Continued)

ITEM	YES	NO	N/A	NARRATIVE
10. The proper placement of animals which are transferred from one cage to another in the same location is checked by the transferer and verified by a responsible person, appropriately documented, and a record of the procedure maintained.				
11. Animal waste and refuse is collected, stored and disposed of in a safe and sanitary manner so as to preclude vermin infestation, odors, and disease hazards.				
12. Animal cages, racks and accessory equipment are cleaned and sanitized at appropriate intervals as recommended in HEW Publication No. (NIH) 74-23 or subsequent revisions.				
13. Storage areas for feed, bedding, supplies, clean cages, and equipment are separate from areas housing the test systems as well as the quarantine and isolation area, and these materials are protected against spoilage, infestation or contamination.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "ANIMAL CARE"
IN THE NARRATIVE PORTION OF THE REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

G. TEST AND CONTROL SUBSTANCES

ITEM	YES	NO	N/A	NARRATIVE
1. Each container for a test and control substance is appropriately labeled and adequately stored to maintain the identity, strength, quality, and purity of said substances.				
2. An appropriately identified reserve sample selected at random from each batch of test and control substance used in a study of more than 4-weeks duration, is taken, stored in an identical immediate container under appropriate storage conditions, and analyzed at the time the batch is depleted, at the termination of the study, or at the expiration date (whichever occurs first) to assure that the identity, quality, strength, purity, and stability conform to established specifications.				
3. If test or control substances are mixed with a carrier prior to administration, each batch of such mixture is tested periodically for the adequacy of the mix to assure uniformity and to determine the concentration of the substance in the mixture. Describe procedures used.				
4. Enough samples of each batch of the mixture are returned to the sponsor for such analysis if the study is a blind study.				
5. Each batch of the test and control substance-carrier mix is tested for stability for at least the length of time between mixing and use and to establish storage conditions and an expiration date.				
6. For each batch of the test and control substance, tests are performed to determine the release from the carrier mix and the results documented.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "TEST AND CONTROL SUBSTANCES" IN THE NARRATIVE PORTION OF THE REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

G. TEST AND CONTROL SUBSTANCES (Continued)

ITEM	YES	NO	N/A	NARRATIVE
7. For each batch of test and control substance mixed with a carrier an appropriately identified reserve sample of each batch of the substance/mixture is taken and retained for the required length of time.				
8. All handling, storage, and disposal of known or suspected chemical carcinogens used as the test substance in a study are treated in accordance with the safety principles set forth in the "Nat'l. Cancer Inst. Safety Standards for Res. Involving Chemical Carcinogens," HEW Pub. No. (NIH) 75-900.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "TEST AND CONTROL SUBSTANCES"
IN THE NARRATIVE PORTION OF THE REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

H. STUDY IMPLEMENTATION AND CONDUCT

ITEM	YES	NO	N/A	NARRATIVE
1. Scientists or other professional persons are available to provide assistance and consultation to subordinates and to handle unforeseen issues.				
2. Specimens are identified by test system number, study number, nature of specimen and date. Explain identification system.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "STUDY IMPLEMENTATION AND CONDUCT IN THE NARRATIVE PORTION OF REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

I. STORAGE AND RETRIEVAL OF RECORDS AND DATA

ITEM	YES	NO	N/A	NARRATIVE
1. The testing facility maintains and retains all raw data, documentation and other information, protocols, specimens, and final reports generated during and as the result of a nonclinical laboratory study, and they are retained in an archive of adequate space and design and are indexed to facilitate their orderly and expedient storage and retrieval.				
2. The archive provides the proper conditions to minimize deterioration of all stored material for as long as they are required to be retained.				
3. The archive contains specific reference to other locations in which documents and specimens may be stored.				
4. Documents and specimens required to be maintained in the archive and not physically present there have appropriate and specific reference to their location filed in the archive.				
5. An individual responsible for the archive is identified.				
6. Only authorized personnel enter the archive and whenever a custodian of the archive is not present the suitable repositories for the documents and specimens are locked.				
7. Whenever the original material is transferred to the sponsor's archive at the sponsor's request at the completion of the study, duplicates of all material required to be in the archive are retained there, when the nature of the material permits.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "STORAGE AND RETRIEVAL OF RECORDS AND DATA" IN THE NARRATIVE PORTION OF THE REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

I. STORAGE AND RETRIEVAL OF RECORDS AND DATA (Continued)

ITEM	YES	NO	N/A	NARRATIVE
8. All material required to be retained in the archive is available for inspection to authorized employees of the Food and Drug Administration.				
9. If the archive has been contracted out to a commercial archive not belonging to the research facility or sponsor, then the name and address of the commercial archive has been provided to the sponsor in the submission of the final study report.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "STORAGE AND RETRIEVAL OF RECORDS AND DATA" IN THE NARRATIVE PORTION OF THE REPORT.

NONCLINICAL LABORATORY INSPECTION REPORT

J. RETENTION OF RECORDS

ITEM	YES	NO	N/A	NARRATIVE
1. All protocols, raw data, specimens, final reports and other required documents pertinent to the conduct of the study, including records and reports of maintenance, cleaning, calibration and inspection of equipment, are stored in an archive, and retained for the specified time.				
2. Curriculum vitae and job descriptions of all personnel engaged in conducting the study are retained for the specified period of time, either in the facility employment records, or the archive; and are available for inspection.				
3. The master schedule sheet, records of inspection or evaluation and status reports of the quality assurance unit are retained for specified period of time.				

INCLUDE ANY OTHER SIGNIFICANT GLP DEVIATIONS CONCERNING "RETENTION OF RECORDS" IN THE NARRATIVE PORTION OF THE REPORT.

APPENDIX B
TOXICOLOGY STUDY
AUDIT GUIDE FORMS
ACUTE STUDIES

FIRST DRAFT

TOXICOLOGY STUDY AUDIT GUIDE

Acute Toxicology Studies

Test:

Audit Dates:

Audit Team:

Laboratory:

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TOXICOLOGY STUDY AUDIT GUIDE

Acute Toxicology Studies

101.0 GOALS OF AUDIT:

1. Assure that study was performed according to established protocol.
2. Assure accuracy of data in final report by review and/or verification of raw data.
3. Assure that study was performed according to Good Laboratory Practices.
4. Assure that the report will be acceptable to the appropriate regulatory agencies, e.g., EPA.
5. Assure the scientific integrity of the report.

Implementation

Will involve answering several questions about the report and the conduct of the study, as well as verification of certain data.

102.0 PERSONNEL:

Questions

1. Were there sufficient numbers of personnel to perform the study in an acceptable manner?

2. What were the qualifications of each person, both professional and technical, involved in the study?

3. Did they have the necessary background (education plus training) and experience to perform their assigned roles in the study?

4. What supervision did they receive?

Verification

1. List all the personnel involved in the conduct or review of the study along with a brief description of their duties or roles in performance of this study.

2. Attach the curricula vitae of each person, both professional and technical, involved in the study.

103.0 PROTOCOL:

Questions

1. Was a specific protocol prepared as a detailed guide to the conduct or performance of this study?

2. If not, why not?
3. Who designed the protocol?
4. Who at Gulf approved the protocol?
5. Were there any written amendments or changes to the protocol?
6. Who approved these changes?
7. Were there any verbal amendments or changes to the protocol?
8. Who approved these changes?
9. Were there any significant deviations from the protocol or its amendments and changes?
10. What were the reasons for these deviations?
11. What was the name and title of the person who authorized these changes?
12. What was the effect of these changes on the outcome or findings of the study?

Verification

1. Attach a copy of the original protocol.

2. List all approved amendments or changes to the original protocol, along with the date the change was approved and the name and title of the person authorizing the change.

3. List any unauthorized protocol deviations found during the review of the raw data, along with the dates of the unauthorized protocol deviations, the appropriate animal number, the manipulation involved, and any stated reason for the deviation.

104.0 DATES:

Questions

1. What are the dates stated in the report for receipt and quarantine of the experimental animals?

2. What are the dates found in the raw data for receipt and quarantine of the experimental animals?

3. Are there any discrepancies between dates in report and raw data? If so, explain.

4. What are the dates for the conduct of the study contained in the final report?

5. What are the dates for the conduct of the study as verified by examination of the raw data?

6. Are there any discrepancies between dates in report and raw data? If so, explain.

7. What was the intended duration of the study?

8. What was the actual duration of the study?

9. Were there any discrepancies between the intended and actual duration of the study? If so, explain.

10. What was the effect of any of these discrepancies on the outcome of the study as a useful tool in predicting the safety of the test material?

Verification

1. List the dates found in the final report for the following:

(a) receipt of experimental animals -

(b) quarantine period -

(c) period of compound administration -

(d) interim necropsies -

(e) terminal necropsy -

2. List the dates found in the raw data for the following:

(a) receipt of experimental animals -

(b) quarantine period -

(c) period of compound administration -

(d) interim necropsies -

(e) terminal necropsy -

3. List any discrepancies found, including: date, animal number, procedure involved, reason for discrepancy, if known.

105.0 TEST MATERIAL CONTROL:

Questions

1. What are the written procedures for handling of the test material?
(Receipt, identification, handling, distribution, storage.)

2. Were these procedures followed? If not, explain.

3. How was the test material administered to the animals?
4. Did the animals receive the correct doses of the test material--as stated in the final report?
5. How was this determined?
6. Were there any discrepancies or errors found in the administration of the test material? If so, explain.
7. What is the effect of these discrepancies on the outcome of the study and its use as a tool in predicting the safety of the test material?

Verification

1. Recalculate the individual doses administered to several animals (3 rodents, 2 rabbits, dogs, etc.) in each group (dose level).
2. List any observed discrepancies in dosage calculations, including: date, animal number, reason for discrepancy, if known.

106.0 FACILITIES:

Questions

1. Were the number and size of the animal rooms used adequate for the performance of the study? If not, explain.

2. Were the supporting laboratories, e.g., necropsy room, compound mixing room, compound storage room, clinical laboratory, adequate for the performance of the study? If not, explain.

3. Were the areas for the employees, e.g., bathrooms, office space, showers, lunchroom, adequate? If not, explain.

4. Are the areas for data storage and retrieval adequate? If not, explain.

Verification

1. Write a brief narrative describing the animal rooms, supporting laboratories, employee areas, and data storage and retrieval systems.

2. List any specific inadequacies observed in the above.

107.0 EQUIPMENT:

Questions

1. Was there adequate and suitable equipment available for the performance of this study? If not, explain.

2. Were the conditions under which the equipment was used proper and adequate for the intended use (e.g., air conditioning for analytical instruments)? If not, explain.

3. Were suitable written procedures for the operation, inspection, maintenance, and calibration available for the equipment used in this study? Were these procedures followed? If not, explain.

4. Were adequate records kept of the inspection, maintenance, and calibration of the equipment used in this study? If not, explain.

Verification

1. List the major pieces of equipment used in the study.

2. List any discrepancies observed in the adequacy of the equipment, the conditions of use, the availability of written procedures for operation, maintenance, inspection, and calibration, as well as inadequacies in the documentation that these procedures were followed.

108.0 ANIMALS:

Questions

1. Does the laboratory breed its own animals or are they obtained from a commercial supplier?

2. What is the name, address, and USDA registration number of the supplier?

3. What were the acceptance criteria used to select the animals for this study?

4. What were the general criteria used to select the animals for this study, e.g., species, strain, sex, number, age, weight range?

5. Were there any deviations from these criteria? If so, explain.

6. What were the laboratory's procedures for quarantine, randomization, identification, and environment?

7. Were there any deviations from these procedures? If so, explain.

8. Was a veterinarian available for care and treatment of diseased or injured animals? Explain the procedure for routine veterinary care.

9. Did any animal on study receive prophylaxis or therapy for injury or disease before or during the study? If so, explain.

10. How did this treatment affect the data generated during the performance of the study.

11. Were species separated by room? If not, explain.

12. Were the transfers of test animals between cages, e.g., for cage cleaning, adequately documented and verified? If not, explain.

13. What were the written procedures for cleaning of cages and racks? Were these followed? If not, explain.

14. What type of bedding was used in the animal cages? Why.

15. Would this type of bedding interfere with the assessment of the safety of the test material? If so, explain.

16. Were the conditions of animal husbandry satisfactory according to USDA and AAALAC standards? If not, explain.

Verification

1. List any deviations from the protocol or standard operating manuals or USDA/AAALAC standards observed in the care and handling of laboratory animals used in this study.

109.0 GENERAL LABORATORY OPERATIONS:**Questions**

1. Were there standard operating procedure manuals available for the routine laboratory procedures used to perform this study? If not, explain.
2. Were records of inspection and calibration of equipment readily available? If not, explain.
3. Were senior scientists available for consultation during the study.

Verification

1. List any deviations from standard written laboratory procedures observed during the course of the inspection.

110.0 STUDY EXECUTION:**Questions**

1. What was the laboratory's plan for collection and verification of data from the study?
2. Was the system for recording documentation and verification of original data adequate? If not, explain.
3. How frequently were the animals observed for any abnormalities? How was data recorded and verified?

4. How frequently were body weight data obtained? How were data recorded and verified?

5. What was the procedure for examination of moribund or dead animals? Were body weights obtained? If not, explain.

6. Was the amount of test material consumed measured? Describe the method. How were the data recorded and verified?

Verification

1. Verify by checking the entry or number in raw data -vs- report for the data collected from several animals (3 rodents, 2 rabbits, dogs, etc.) per group (dose level) for: daily observations, body weight, test material consumption, terminal body weights, mortality data, and any other data generated during the course of the study.

2. Recalculate means from several animals (3 rodents, 2 rabbits, dogs, etc.) per group and compare the report with raw data.

3. Recalculate several statistical computations in each group (dose level) of animals (3 for rodents, 2 for rabbits, dogs, etc.) to check accuracy of calculations. Be sure methodology is same as stated in report.

111.0 NECROPSIES (GROSS PATHOLOGY):

Questions

1. Were necropsies performed on all animals? If not, explain.
2. Were decomposed (autolyzed animals examined? If not, explain.
3. Were all necropsies performed under the supervision of a pathologist? If not, explain.
4. What organs were examined? Who made the observations?
5. Were all lesions in any tissue or organ system described? If not, explain.
6. Were the necropsy records accurate? If not, explain.

Verification

1. Examine necropsy sheets and list the missing data by name, animal number, and group number.

2. Give reasons for their absence if known.
3. Check mortality raw data -vs- report and list discrepancies.

4. Check necropsy sheet observations -vs- report (frequency table) and note discrepancies.

112.0 REPORT WRITING:

Questions

1. Who prepared the final report?
2. Was the report submitted as a "draft" before final version was submitted to Gulf.
3. Did the report accurately account for all animals originally placed on study?
4. If not, what discrepancies were noted and in what part of the study?
5. What is the potential impact of these errors on the scientific integrity of the study.

Verification

1. If all animals that were placed on study were not accounted for in the final report and raw data, list the individual animal numbers of these lost animals.
2. Itemize any corrections to the final report submitted, as amendments, by Gulf.

113.0 STATISTICS:Questions

1. What statistical evaluations of data were made? Please list type of data and statistical test(s) performed?
2. Was a computer used to perform the statistical tests? If so, describe the system, briefly.
3. What was the value of P? Was this consistent for all statistical tests? If not, explain.
4. What was the rounding convention used?

Verification

1. Several of the statistical data (10 calculations) should be re-computed at Gulf as a means of verification. Thus sufficient copies of raw data should be made to aid in verification.

114.0 DATA STORAGE AND RETRIEVAL:Questions

1. Was the raw data for this study readily available for inspection? If not, explain.

2. What is the procedure for storage of raw data at the end of a study? Where and in what manner is it stored? Is it bound in book form? If so, how and by whom?

3. Who has access to this raw data? In what manner is access controlled? Who is responsible for the archives (record storage area)?

4. Are written procedures available for control of data storage and retrieval?

5. Are they adequate? If not, explain.

6. Are they being followed? If not, explain.

7. What is the length of time raw data is stored by the laboratory?

8. If raw data is to be destroyed, is the sponsor notified for permission?

How?

Verification

1. List any deficiencies seen in system for data storage and retrieval.

APPENDIX C
TOXICOLOGY STUDY
AUDIT GUIDE FORMS:
GENERAL TOXICOLOGY
ADDENDUM FOR REPRODUCTIVE STUDIES
ADDENDUM FOR INHALATION STUDIES

SECOND DRAFT

TOXICOLOGY STUDY AUDIT GUIDE

General Toxicology

Test:

Audit Dates:

Audit Team:

Laboratory:

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TOXICOLOGY STUDY AUDIT GUIDE

General Toxicology

101.0 - GOALS OF AUDIT:

1. Assure that study was performed according to established protocol.
2. Assure accuracy of data in final report by review and/or verification of raw data.
3. Assure that study was performed according to good laboratory practices.
4. Assure that the report will be acceptable to the appropriate regulatory agencies, e.g., EPA.
5. Assure the scientific integrity of the report.

Implementation

Will involve answering several questions about the report and the conduct of the study, as well as verification of certain data.

102.0 - PERSONNEL:

Questions

1. Were there sufficient numbers of personnel to perform the study in an acceptable manner?

2. What were the qualifications of each person, both professional and technical, involved in the study?

3. Did they have the necessary background (education plus training) and experience to perform their assigned roles in the study?

4. What supervision did they receive?

Verification

1. List all the personnel involved in the conduct or review of the study along with a brief description of their duties or roles in performance of this study.

2. Attach the curricula vitae of each person, both professional and technical, involved in the study.

103.0 - PROTOCOL:**Questions**

1. Was a specific protocol prepared as a detailed guide to the conduct or performance of this study?

2. If not, why not?

3. Who designed the protocol?

4. Who at Gulf approved the protocol?

5. Were there any written amendments or changes to the protocol?

6. Who approved these changes?

7. Were there any verbal amendments or changes to the protocol?

8. Who approved these changes?

9. Were there any significant deviations from the protocol or its amendments and changes?

10. What were the reasons for these deviations?

11. What was the name and title of the person who authorized these changes.

12. What was the effect of these changes on the outcome or findings of the study?

Verification

1. Attach a copy of the original protocol.

2. List all approved amendments or changes to the original protocol, along with the date the change was approved and the name and title of the person authorizing the change.

3. List any unauthorized protocol deviations found during the review of the raw data, along with the dates of the unauthorized protocol deviations, the appropriate animal number, the manipulation involved, and any stated reason for the deviation.

104.0 - DATES:

Questions

1. What are the dates stated in the report for receipt and quarantine of the experimental animals?

2. What are the dates found in the raw data for receipt and quarantine of the experimental animals?

3. Are there any discrepancies between dates in report and raw data? If so, explain.

4. What are the dates for the conduct of the study contained in the final report?

5. What are the dates for the conduct of the study as verified by examination of the raw data?

6. Are there any discrepancies between dates in report and raw data? If so, explain.

7. What was the intended duration of the study?

8. What was the actual duration of the study?

9. Were there any discrepancies between the intended and actual duration of the study? If so, explain.

10. What was the effect of any of these discrepancies on the outcome of the study as a useful tool in predicting the safety of the test material?

Verification

1. List the dates found in the final report for the following:

a) receipt of experimental animals -

b) quarantine period -

c) period of compound administration -

d) interim necropsies -

e) terminal necropsy -

2. List the dates found in the raw data for the following:

a) receipt of experimental animals -

- b) quarantine period -
- c) period of compound administration -
- d) interim necropsies -
- e) terminal necropsy -

3. List any discrepancies found, including: date, animal number, procedure involved, reason for discrepancy, if known.

105.0 - TEST MATERIAL CONTROL:

Questions

1. What are the written procedures for handling of the test material?
(Receipt, identification, handling, distribution, storage.)

2. Were these procedures followed? If not, explain.

3. What are the written procedures for establishing the identity, purity, and stability of each batch of test material used in the study?

4. Were these followed? If not, explain.

5. How was the test material administered to the animals?

6. Was there an adequate program for testing adequacy of mixing, concentration in dosage form, stability of dosage form, and bioavailability of the test material? If not, explain.

7. Describe the procedure for taking samples of the test material for analysis. Was this procedure followed? If not, explain.

8. If test material was added to diet or drinking water, describe the procedure for taking samples (method, frequency) for analysis. Was this procedure followed? If not, explain.

9. Where was the analysis of the test material (including diet/drinking water admixtures) performed?

10. Did the animals receive the correct doses of the test material--as stated in the final report?

11. How was this determined?

12. Were there any discrepancies or errors found in the administration of the test material? If so, explain.

13. What is the effect of these discrepancies on the outcome of the study and its use as a tool in predicting the safety of the test material?

Verification

1. Recalculate the individual doses administered to several animals (3 rodents, 2 rabbits, dogs, etc.) in each group (dose level).

2. List any observed discrepancies in dosage calculations, including: date, animal number, reason for discrepancy, if known.

106.0 - FACILITIES:

Questions

1. Were the number and size of the animal rooms used adequate for the performance of the study? If not, explain.

2. Were the supporting laboratories, e.g., necropsy room, compound mixing room, compound storage room, clinical laboratory, adequate for the performance of the study? If not, explain.

3. Were the areas for the employees, e.g., bathrooms, office space, showers, lunchroom, adequate? If not, explain.

4. Are the areas for data storage and retrieval adequate? If not, explain.

Verification

1. Write a brief narrative describing the animal rooms, supporting laboratories, employee areas, and data storage and retrieval systems.

2. List any specific inadequacies observed in the above.

107.0 - EQUIPMENT:

Questions

1. Was there adequate and suitable equipment available for the performance of this study? If not, explain.

2. Were the conditions under which the equipment was used proper and adequate for the intended use (e.g., air conditioning for analytical instruments)? If not, explain.

3. Were suitable written procedures for the operation, inspection, maintenance, and calibration available for the equipment used in this study? Were these procedures followed? If not, explain.

4. Were adequate records kept of the inspection, maintenance, and calibration of the equipment used in this study? If not, explain.

Verification

1. List the major pieces of equipment used in the study.

2. List any discrepancies observed in the adequacy of the equipment, the conditions of use, the availability of written procedures for operation, maintenance, inspection, and calibration, as well as inadequacies in the documentation that these procedures were followed.

108.0 - ANIMALS:

Questions

1. Does the laboratory breed its own animals or are they obtained from a commercial supplier?

2. What is the name, address and USDA registration number of the supplier?

3. What were the acceptance criteria used to select the animals for this study?

4. What were the general criteria used to select the animals for this study, e.g., species, strain, sex, number, age, weight range?

5. Were there any deviations from these criteria? If so, explain.

6. What were the laboratories' procedures for quarantine, randomization, identification, and environment?

7. Were there any deviations from these procedures? If so, explain.

8. Was a veterinarian available for care and treatment of diseased or injured animals? Explain the procedure for routine veterinary care.

9. Did any animal on study receive prophylaxis or therapy for injury or disease before or during the study? If so, explain.

10. How did this treatment affect the data generated during the performance of the study?

11. Was there more than one study per room? If so, explain.

12. Were species separated by room? If not, explain.

13. Were the transfers of test animals between cages, e.g., for cage cleaning, adequately documented and verified? If not, explain.

14. What were the written procedures for cleaning of cages and racks? Were these followed? If not, explain.

15. Was food and drinking water analyzed for content and purity? What was the frequency? What were the results? If this was not done, explain.

16. What type of bedding was used in the animal cages? Why?

17. Would this type of bedding interfere with the assessment of the safety of the test material? If so, explain.

18. Were the conditions of animal husbandry satisfactory according to USDA and AAALAC standards? If not explain.

Verification

1. List any deviations from the protocol or standard operating manuals or USDA/AAALAC standards observed in the care and handling of laboratory animals used in this study.

109.0 - GENERAL LABORATORY OPERATIONS:**Questions**

1. Were there standard operating procedure manuals available for the routine laboratory procedures used to perform this study? If not, explain.

2. Were records of inspection and calibration of equipment readily available? If not, explain.

3. Were senior scientists available for consultation during the study?

Verification

1. List any deviations from standard written laboratory procedures observed during the course of the inspection.

110.0 - STUDY EXECUTION:**Questions**

1. What was the laboratory's plan for collection and verification of data from the study?

2. Was the system for recording, documentation and verification of original data adequate? If not, explain.

3. How frequently were the animals observed for any abnormalities? How was data recorded and verified?

4. How frequently were body weight data obtained? How were data recorded and verified?

5. How frequently were food consumption data obtained? How were data recorded and verified?

6. Were the animals palpated for tissue masses? How frequently?
By whom?

7. What clinical studies (hematology, urinalysis, clinical chemistry) were performed on the test animals? How many animals per group per sex were examined in this manner? Were the animals fasted before blood and urine samples were taken? How long?

8. Were background data on this strain and age of animal available for clinical studies, etc.? Was this data used in verification of study data? How?

9. What laboratory methods were used to perform these tests? Were standard procedure manuals available listing the manner in which these tests were performed? How were data recorded and verified?

10. What was the procedure for examination of moribund or dead animals? Were blood and tissue samples obtained? Were body and organ weights obtained? If not, explain.

11. Was the amount of test material consumed measured? Describe the method. How were the data recorded and verified?

12. Were ophthalmological examinations performed on the animals? How often? By whom? How were the data recorded and verified?

13. What was the procedure for identifying specimens (blood, urine, tissue samples) taken during the course of the study (e.g., unique number, study number, date, etc.)?

14. Were these procedures followed? If not, explain.
15. To whom were the data sent and at what intervals?
16. Who checked the accuracy? Describe the procedure in detail.
17. Was a life table method always used to evaluate the results?
If not, what other method was used?

Verification

1. Verify by checking the entry or number in raw data vs. report for the data collected from several animals (3 rodents, 2 rabbits, dogs, etc.) per group (dose level) for: daily observations, body weight, food consumption, test material consumption, clinical tests (hematology, urinalysis, clinical chemistry), organ weights (absolute and relative), terminal body weights, tumor frequency, mortality data, and any other data generated during the course of the study.

2. Recalculate means from several animals (3 rodents, 2 rabbits, dogs, etc.) per group and compare the report with raw data.

3. Recalculate several statistical computations in each group (dose level) of animals (3 for rodents, 2 for rabbits, dogs, etc.) to check accuracy of calculations. Be sure methodology is same as stated in report.

111.0 - NECROPSIES (GROSS PATHOLOGY):**Questions**

1. Were necropsies performed on all animals? If not, explain.
2. Were decomposed (autolyzed) animals at least examined for tumors? If not, explain.
3. Were all necropsies performed under the supervision of a pathologist? If not explain.
4. What organs were examined? Who made the observations?
5. Which organs were weighed? Were there any deviations from protocol in this regard? If so, explain.
6. Were all lesions in any tissue or organ system described? If not explain.

7. What was the procedure for assigning specific organs or tissues to a specific slide number for a particular species? Was this procedure followed? If not, explain.

8. What wet tissues, tissue blocks and slides were preserved? By whom? How are they identified?

9. Where is this information recorded?

10. Where are the slides stored?

11. Were the necropsy records accurate? If not, explain.

Verification

1. Examine necropsy sheets and list the missing tissues lost at necropsy by name, animal number, and group number.

2. Give reasons for their absence if known.
3. Check the list of tissues examined by pathologist and if it differs from protocol, record discrepancies (including animal number and missing tissue).
4. Record missing tissues from pathology report and compare with list prepared from examination of necropsy sheets. List discrepancies.
5. Check mortality raw data vs. report and list discrepancies.
6. Check necropsy sheet observations vs. report (frequency table) and note discrepancies.

112.0 - HISTOPATHOLOGY:**Questions**

1. Who prepared the slides? Where was this done? How was the data recorded? Was this record saved?
2. What was the procedure used to assure that all tissues taken at necropsy were examined microscopically? Attach a copy of the missing tissue list from the Histology Laboratory.
3. What stains were used routinely? If special stains were used, what were they? Who authorized their use and why?
4. Who examined the slides?
5. When were the slides examined in relation to completion of the gross pathology?

6. Were all lesions and/or tumors noted on necropsy sheets examined microscopically? If not, explain.

7. Were the histopathology records accurate?

8. If more than one pathologist read the slides, were there differences among the readers? If so, explain.

9. Is the pathologist's summary of the histopathology data accurate? If not, explain.

Verification

1. List missing tissues found in Histology Laboratory.

2. Examine data and list discrepancies found in histopathology records.

3. Obtain all of the microscopic slides used to prepare the pathology report. Have a pathologist in Gulf's employ check several slides per dose level and sex (3 slides for rodents, 2 slides for rabbits, dogs, etc.) against the original pathologist's report. List any discrepancies observed.

113.0 - REPORT WRITING:**Questions**

1. Who prepared the final report?
2. Was the report submitted as a "draft" before final version was submitted to Gulf?
3. Did the report accurately account for all animals originally placed on study?
4. If not, what discrepancies were noted and in what part of the study?
5. What is the potential impact of these errors on the scientific integrity of the study?
6. Are the reports (signed and dated) of all individual scientists other than the project director (e.g., pathologist, ophthalmologist, cardiologist, analytical chemist) included in the appendices of the final report?
7. If not, explain any discrepancies.

Verification

1. If all animals that were placed on study were not accounted for in the final report and raw data, list the individual animal numbers of these lost animals.

2. Itemize any corrections to the final report submitted, as amendments, by Gulf.

114.0 - STATISTICS

Questions

1. What statistical evaluations of data were made? Please list type of data and statistical test(s) performed?

2. Was a computer used to perform the statistical tests? If so, describe the system, briefly.

3. What was the value of P? Was this consistent for all statistical tests? If not, explain.

4. What was the rounding convention used?

Verification

1. Several of the statistical data (10 calculations) should be re-computed at Gulf as a means of verification. Thus sufficient copies of raw data should be made to aid in verification.

115.0 - DATA STORAGE AND RETRIEVAL:

Questions

1. Was the raw data for this study readily available for inspection? If not, explain.

2. What is the procedure for storage of raw data at the end of a study? Where and in what manner is it stored? Is it bound in book form? If so, how and by whom?

3. Who has access to this raw data? In what manner is access controlled? Who is responsible for the archives (record storage area)?

4. Are written procedures available for control of data storage and retrieval?

5. Are they adequate? If not, explain.

6. Are they being followed? If not, explain.

7. How are wet tissues, slides, and tissue blocks stored? Where and in what manner?

8. Who has access to these and how is this access controlled?
9. What is the length of time raw data is stored by the laboratory?
10. If raw data is to be destroyed, is the sponsor notified for permission? How?
11. How long are wet tissue slides and tissue blocks stored?
12. If these are to be destroyed, is the sponsor notified for permission? How?

Verification

1. List any deficiencies seen in system for data storage and retrieval.

ADDENDUM FOR REPRODUCTIVE STUDIES

ADDENDUM TO Q.A. FORMS FOR VALIDATING
REPRODUCTIVE TOXICOLOGY STUDIES:

Study Examined:

By:

Laboratory:

Date:

1.0 ANIMAL DATA:

- 1.1 How were pregnancies produced (e.g., purchase of timed-pregnant, bred in laboratory)?
- 1.2 If animals were bred in the laboratory, how many pregnant females were produced per breeding? How many breeding periods were necessary?
- 1.3 How many females were placed with how many males to induce pregnancy? How long were they left together?
- 1.4 How was pregnancy determined (plug, smear, etc.)?
- 1.5 What was length of gestation period? How was this determined?
- 1.6 Were pups allowed to mature or were they sacrificed at birth or weaning?
- 1.7 If so, what method was employed?

1.8 At what age were the pups weaned?

1.9 List any discrepancies observed.

2.0 MEASUREMENTS:

2.1 What measurements were made on pregnant dams? How frequently?

2.2 List any discrepancies observed between protocol and raw data.

2.3 What measurements were made on fetuses? How frequently?

2.4 List any discrepancies observed between protocol and raw data.

2.5 What statistical procedures were performed on litter data? Why were these procedures chosen? (Give references for each test that is not routine.)

- 2.6 Was histopathology required by protocol? If not, were tissues taken and stored? What tissues?
- 2.7 If brother/sister matings are to be avoided, how was this accomplished?
- 2.8 List any discrepancies observed.

3.0 MISCELLANEOUS:

- 3.1 If fetuses were examined, describe the technique used.
- 3.2 How were fetuses stored? How were they labeled?
- 3.3 Does laboratory have adequate data on background incidence of anomalies for strain of animals used? If so, attach.
- 3.4 List any discrepancies observed.

ADDENDUM FOR INHALATION STUDIES

ADDENDUM TO Q.A. FORMS FOR VALIDATING
INHALATION TOXICOLOGY STUDIES

Study Examined:

By:

Laboratory:

Date:

1.0 EQUIPMENT:

- 1.1 Were inhalation chambers used in performance of the study.
What size? How many?

- 1.2 Who manufactured the chambers?

- 1.3 Was chamber exhaust scrubbed, filtered, or merely vented to the
atmosphere? How was this done? How often were filters changed?

- 1.4 What was source of air supply to chambers (outside, laboratory, etc.)?

- 1.5 What was the flow rate of air through the chamber?

1.6 Were pans placed beneath animal cages to collect urine and feces?
Did this interfere with distribution of test material?

1.7 Was there an alarm system to monitor power failure in chamber pumps?
How is alarm system monitored?

1.8 Were chambers of adequate size, design, construction, and number for
conduct of study?

1.9 List any discrepancies.

2.0 ANIMALS:

2.1 How were animals caged during exposure period, i.e., gang or individual?
If gang-housed, how many animals per cage?

2.2 Where were animals kept when exposure period was completed? How
many animals per cage?

2.3 What was duration of exposure period (hours per day, days per week)?

- 2.4 How was response of animals to test compound measured? How frequently?
By whom?
- 2.5 How many layers of animals were in chamber at one time?
- 2.6 Did this interfere with dispersion of test material in chamber?
- 2.7 Was an analytical traverse of the test material in the chamber performed?
How was this done? Were the animals in the chamber at the time?
- 2.8 List any discrepancies.

3.0 TEST MATERIAL:

- 3.1 In what form was test material administered to animals, e.g., dust,
vapor, aerosol, etc.?

- 3.2 How was dust, vapor, etc., generated? What equipment was used?
- 3.3 What was particle size of test material?
- 3.4 How was particle size measured? How frequently?
- 3.5 Were both nominal and actual concentrations of test material determined?
If nominal only, why?
- 3.6 How was nominal concentration determined? How frequently?
- 3.7 How was actual concentration determined? How frequently?
- 3.8 Is test material explosive? If so, what precautions were taken to prevent explosion?
- 3.9 List any discrepancies.

4.0 MEASUREMENTS:

4.1 Were respiratory physiology studies performed on test animals? How frequently?

4.2 List respiratory parameters measured.

4.3 What equipment was used?

4.4 What other parameters were measured? How frequently?

4.5 Were staff and equipment adequate to perform these studies?

4.6 List any discrepancies.