MUTAGENICITY OF 13 NIOSH PRIORITY COMPOUNDS

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ABSTRACT

THIRTEEN COMPOUNDS WERE INVESTIGATED FOR MUTAGENIC POTENTIAL. FIVE MUTAGEN SCREENING TESTS WERE USED. THEY WERE THE: DOMINANT LETHAL MAMMALIAN (MOUSE) ASSAY (DL); *DROSOPHILA* RECESSIVE LETHAL TEST (DRL); BONE MARROW CYTOLOGY ASSAY (BMC); SPERM MORPHOLOGY TEST (SM); AND UNSCHEDULED DNA SYNTHESIS (UDS) USING AN *IN VITRO* HUMAN CELL LINE.

THE DL, SM, AND BMC ASSAYS EMPLOYED TWO LEVELS OF INHALATION EXPOSURES, EACH LASTING 7 HOURS PER DAY FOR 5 CONSECUTIVE DAYS. IN ADDITION, THE BMC ASSAY USED SINGLE 7-HR EXPOSURES TO THE SAME LEVELS USED IN THE MULTIPLE DAY REGIMEN; ALL EXPOSURES WERE FOLLOWED BY SAMPLING AT 6, 24, AND 48 HRS. THE UDS TEST USED A 3-HR EXPOSURE OVER A WIDE RANGE OF 8 CONCENTRATIONS. THE DRL ASSAY USED EITHER ONE OR TWO CONCENTRATIONS OVER A PERIOD OF 15 MINUTES TO UP TO 7 HOURS DEPENDING UPON TOXICITY.

THERE WAS NO EVIDENCE THAT GENETIC DAMAGE WAS INDUCED IN SEVEN OF THE CHEMICALS STUDIED (3-CHLOROPROPENE, CYCLOHEXANONE, METHYL BROMIDE, N,N-DIMETHYL ACETAMIDE, N,N-DIMETHYL FORMAMIDE, N-METHYL DICYCLOHEXYLAMINE, AND 2-NITROPROPANE). BIS(2-METHOXYETHYL) ETHER AND 2-METHOXYETHANOL, BOTH GLYCOL ETHER DERIVATIVES, DEMONSTRATED A STRONG ANTI-FERTILITY EFFECT AND LOW-LEVEL GENETIC TOXICITY. STYRENE OXIDE SHOWED A SIMILAR

ANTI-FERTILITY EFFECT BUT WITH NO EVIDENCE OF GENETIC TOXICITY. BUTYLENE OXIDE RESULTS WERE NEGATIVE WITH THE EXCEPTION OF A QUESTIONABLE FINDING IN THE BMC ASSAY. HEXACHLORO-1,3-BUTADIENE ALSO SHOWED A QUESTIONABLE BMC RESULT AS WELL AS AN UNCLEAR EFFECT IN THE DL ASSAY. 1,1,2,2-TETRACHLOROETHANE SHOWED "BORDERLINE" RESULTS IN THE BMC AND SM ASSAYS.

METHODS

DOMINANT LETHAL (DL) TEST IN MALE RATS

THE OBJECT OF THIS TEST WAS TO EVALUATE *IN UTERO* MORTALITY IN THE OFFSPRING OF MALE RATS TREATED WITH THE TEST CHEMICAL.

TEN MALE RATS (CD STRAIN DERIVED FROM SPRAGUE-DAWLEY) WERE TREATED OVER A 5-DAY PERIOD FOR EACH OF FOUR TREATMENT GROUPS (THREE INHALATION EXPOSURES, INCLUDING AN AIR-ONLY CONTROL, AT 7 HOURS/DAY; AND A POSITIVE CONTROL GROUP DOSED ORALLY OVER THE SAME 5 CONSECUTIVE DAYS). ON THE FIFTH DAY, EACH TREATED MALE WAS INTRODUCED TO 2 FEMALE CD RATS. THESE FEMALES WERE KILLED 14 DAYS AFTER THE ASSUMED DATE OF FERTILIZATION (17 DAYS AFTER CAGING THE FEMALES WITH THE MALES). THEIR OVARIES WERE EXAMINED FOR CORPORA LUTEA WHICH WERE COUNTED AND RECORDED. THE UTERI WERE OPENED AND EXAMINED FOR LIVE IMPLANTATIONS, EARLY DEATHS AND LATE DEATHS. LIVE IMPLANTATIONS WERE RECOGNIZED AS RAT FETUSES NORMALLY DEVELOPED FOR APPROXIMATELY DAY 14 OF GESTATION AND WITH A VASCULATURE WHICH HAD CLEARLY BEEN FUNCTIONING UNTIL AT LEAST MATERNAL DEATH. A LATE DEATH WAS DIAGNOSED AS A FETUS WHERE ORGANOGENESIS HAD OCCURRED, BUT WAS NOW BLOODLESS DUE TO DEATH OF THE FETUS WITHIN THE LAST 2

DAYS OF INTRA-UTERINE EXISTENCE. AN EARLY DEATH WAS DIAGNOSED AS A POINT OF UTERINE REACTION TO AN IMPLANTING BLASTULA. THIS WAS SEEN AS A SMALL RAISED SPOT ALONG THE LINE OF IMPLANTATIONS.

SUBSEQUENTLY, THE MALES WERE INTRODUCED TO 2 NEW FEMALES EACH WEEK FOR THE NEXT NINE WEEKS WITH REPETITION OF THE ABOVE TASKS PERFORMED ON THE FEMALES.

RESULTS WERE COMPARED WITH POSITIVE CONTROL VALUES (ETHYL METHANE SULPHONATE - 100 MG/KG DOSED ORALLY FOR 5 CONSECUTIVE DAYS) AND WITH NEGATIVE CONTROL VALUES (AIR ONLY IN INHALATION CHAMBERS--7 HOURS/DAY FOR 5 CONSECUTIVE DAYS).

SPERM MORPHOLOGY (SM) TEST
IN MICE

THE OBJECT OF THIS TEST WAS TO EVALUATE SPERM FOR MORPHOLOGICAL ABNORMALITIES FOLLOWING TREATMENT OF MALE MICE TO THE TEST CHEMICALS.

$B6C3F_1$ HYBRID MICE WERE TREATED OVER A 5-DAY PERIOD FOR EACH OF FOUR TREATMENT GROUPS (THREE INHALATION EXPOSURES, INCLUDING AN AIR-ONLY CONTROL, AT 7 HOURS PER DAY; AND A POSITIVE CONTROL GROUP DOSED ORALLY OVER THE SAME 5 CONSECUTIVE DAYS). THE MICE WERE KILLED 5 WEEKS AFTER THE LAST TREATMENT DAY. THE SEMINAL DUCTS OF THE TESTES WERE DISSECTED OUT AND THE CAUDA EPIDIDYMIDES WERE REMOVED AND FINELY MINCED. FROM THIS, A SPERM SUSPENSION WAS FILTERED AND PROCESSED THROUGH A SERIES OF CENTRIFUGATIONS, AND THEN STAINED WITH EOSIN AND MOUNTED ON MICROSCOPE SLIDES. THESE SLIDES WERE EXAMINED UNDER A LIGHT MICROSCOPE WHERE SPERM WERE SCORED BY PLACING THEM INTO EITHER A "NORMAL" OR "ABNORMAL" CATEGORY. A TOTAL OF 10,000 SPERM WERE EXAMINED FOR EACH TREATMENT GROUP. ABNORMAL SPERM WERE DIVIDED INTO 5 SUBCATEGORIES:

A. HOOK UPTURNED OR ELONGATED

B. BANANA-SHAPED

C. AMORPHOUS HEAD

D. ABNORMAL TAIL (SHARP, 180^{0} ANGLE OR TIGHT COILING ONLY)

E. MISCELLANEOUS (THESE COULD INCLUDE MULTIPLE TAILS, DOUBLE HEADS, TWISTED NECK, FILAMENTOUS MIDPIECE, ENLARGED MID-PIECE, PLIER TYPE)

THE FOLLOWING SAMPLES WERE NOT SCORED: SEPARATED TAILS AND HEADS; CLUMPS OF SPERM; SPERM ORIENTED SO THAT THE HOOK COULD NOT BE SEEN; SPERM PARTIALLY MASKED BY ANY REMAINING STAIN DROPLETS.

RESULTS WERE COMPARED WITH POSITIVE CONTROL VALUES (ETHYL METHANE SULPHONATE--100 TO 200 MG/KG DOSED ORALLY FOR 5 CONSECUTIVE DAYS) AND WITH NEGATIVE CONTROL VALUES (AIR ONLY IN INHALATION CHAMBERS--7 HOURS/DAY FOR 5 CONSECUTIVE DAYS).

RECESSIVE LETHAL TEST
IN DROSOPHILA (DRL)

THE BASC OR MULLER-5 TEST WAS USED. IN THIS TEST, RECESSIVE LETHAL MUTATIONS INDUCED IN THE X-CHROMOSOMES OF TREATED MALE GAMETES (OREGON K STRAIN) ARE DETECTED IN THE F2 GENERATION BY THE ABSENCE OF WILD-TYPE MALES IN THE PROGENY OF INDIVIDUAL GAMETES. F3 GENERATION FLIES WERE ALSO OBSERVED SINCE THIS ALLOWS THE DETECTION OF MOSAICS OR DELAYED MUTATIONS WHICH MAY NOT APPEAR IN THE F2 GENERATION.

THREE-DAY-OLD MALES WERE EXPOSED IN A GLASS VESSEL THROUGH WHICH THE TEST ATMOSPHERES WERE PASSED AT THE REQUIRED CONCENTRATIONS. THE LENGTH OF EXPOSURE IN THE MAIN TEST WAS DETERMINED BY PERFORMING A DOSE-RESPONSE ACUTE TOXICITY TEST IN THE WEEK PRIOR TO THE MAIN EXPOSURE.

APPROXIMATELY 100 TREATED MALES WERE MATED INDIVIDUALLY TO TWO VIRGIN MULLER-5 FEMALES ON THE MORNING FOLLOWING THE DAY OF EXPOSURE. EACH MALE WAS REMATED TO TWO MORE VIRGINS 3 DAYS, AND AGAIN, 8 DAYS AFTER THE FIRST MATING. ALL MATINGS CEASED ON DAY 11. THESE 3 BROODS ENSURED THAT SPERM TREATED AT ALL STAGES OF SPERMATOGENESIS WERE TESTED. EMERGENCE OF F2 GENERATION FLIES VIALS FROM THE PUPAE BEGAN ABOUT 10 DAYS AFTER MATING. MATINGS FOR THE F3 GENERATION WERE SET UP 1-4 DAYS

LATER BY MATING BROTHERS WITH SISTERS. BETWEEN 400 TO 600 VIALS, EACH REPRESENTING A SINGLE EXPOSED X-CHROMOSOME, WERE SET UP FOR EACH OF THREE F2 BROODS AND FOR A SINGLE F3 BROOD.

EXPERIMENTS WERE NORMALLY SCORED 11-14 DAYS AFTER ESTABLISHING THE F2 OR F3 CROSSES. VIALS WERE EXAMINED BY EYE AND SCORED AS NON-LETHAL IF 2 OR MORE WILD-TYPE MALES WERE SEEN. IF THESE WERE NOT SEEN THE FLIES WERE SHAKEN OUT ONTO A CARBON DIOXIDE PERMEATED PAD AND EXAMINED UNDER THE MICROSCOPE. VIALS IN WHICH THERE WERE NO WILD-TYPE MALES AND 8 OR MORE M-5 MALES WERE CHECKED FOR THE PRESENCE OF HETEROZYGOUS (M-5/OREGON K) FEMALES AND SCORED AS RECESSIVE LETHALS IF THESE WERE PRESENT.

RESULTS WERE COMPARED WITH POSITIVE CONTROL VALUES (0.4% V/V ETHYL METHANE SULPHONATE IN A SUCROSE FEEDING SOLUTION--5-HOUR EXPOSURE) AND WITH NEGATIVE CONTROL VALUES (AIR ONLY).

UNSCHEDULED DNA SYNTHESIS ASSAY (UDS)

UNSCHEDULED DNA SYNTHESIS, FOLLOWING TREATMENT WITH THE TEST COMPOUNDS, WAS MEASURED IN HUMAN EMBRYONIC INTESTINAL CELLS (OBTAINED FROM FLOW LABORATORIES, IRVINE, SCOTLAND). THE OBJECTIVE WAS TO EVALUATE THE AMOUNT OF DNA DAMAGE UPON THE CELLS BY MEASURING THE AMOUNT OF TRITIATED (^{3}H) THYMIDINE (PROVIDED TO THE CELLS) FOLLOWING TREATMENT WITH EACH TEST CHEMICAL. HIGHER LEVELS OF THYMIDINE INCORPORATION WOULD REFLECT GREATER CELL DAMAGE.

TREATMENT INVOLVED COLLECTING AND SUSPENDING THE CELLS OVER AN INCREASING RANGE OF 8 CONCENTRATIONS IN A DULBECCO'S MINIMUM ESSENTIAL MEDIUM (DMEM) CONTAINING REQUIRED NUTRIENTS, ACTIVATION COMPONENTS, AND THE ^{3}H THYMIDINE. EACH TEST CHEMICAL WAS DISSOLVED IN AN APPROPRIATE SOLVENT AND ADDED TO THE CELL CULTURE FOR A 3-HOUR INCUBATION PERIOD. CULTURES WERE DIVIDED INTO TWO GROUPS; ONE WITH 100 µL OF AN "S-9 MIX" (LIVER FRACTION FOR SUPPLEMENTED METABOLIC ACTIVATION CAPABILITIES), AND ONE WITHOUT. EACH GROUP CONSISTED OF A PETRI DISH CONTAINING 3 WELLS WITH COVERSLIPS (CONTAINING 100,000 CELLS PER WELL). FOLLOWING A RINSING PROCEDURE AND FURTHER INCUBATION, THE CELLS WERE PROCESSED FOR AUTORADIOGRAPHY AND STAINED AND MOUNTED ON MICROSCOPE SLIDES. THE DATA WERE RECORDED AS THE AVERAGE NET GRAIN COUNTS ± STANDARD DEVIATION.

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RESULTS WERE COMPARED WITH POSITIVE CONTROL VALUES (2-AMINOANTHRACENE USED WITH S-9 ADDITION, AND 4-NITROQUINOLINE-N-OXIDE WITHOUT S-9 ADDITION) AND NEGATIVE (VEHICLE--DIMETHYLSULPHOXIDE) CONTROL VALUES.

BONE MARROW CYTOLOGY (BMC)

THIS ASSAY EVALUATED GROSS CHROMOSOMAL DAMAGE IN RATS FOLLOWING TREATMENT WITH THE TEST CHEMICAL. 10 MALE AND 10 FEMALE RATS (CD STRAIN DERIVED FROM SPRAGUE-DAWLEY) WERE TREATED OVER A 5-DAY PERIOD FOR EACH OF FOUR TREATMENT GROUPS (THREE INHALATION EXPOSURES, INCLUDING AN AIR-ONLY CONTROL, AT 7 HOURS PER DAY; AND A POSITIVE CONTROL GROUP DOSED ORALLY OVER THE SAME 5 CONSECUTIVE DAYS). IN ADDITION, A SINGLE 7-HOUR EXPOSURE WAS PERFORMED AT EACH OF THE TWO TEST CONCENTRATIONS.

THE PROCEDURE INVOLVED INJECTING EACH RAT WITH COLCHICINE 4 HOURS AFTER ADMINISTERING THE LAST DOSE OF THE TEST CHEMICAL. TWO HOURS LATER THE RAT WAS KILLED AND THE BONE MARROW OF ONE FEMUR WAS REMOVED AND ASPIRATED INTO TUBES CONTAINING HANK'S BALANCED SALT SOLUTION (HBSS) AND LITHIUM HEPARIN. THE CELL SUSPENSION WAS CENTRIFUGED, THE SUPERNATANT DISCARDED AND REPLACED WITH FRESH HBSS. THE CELLS WERE SUSPENDED, THEN CENTRIFUGED AGAIN AND THE SUPERNATANT DISCARDED. PREHEATED (37^0 C) 0.075 M KCL WAS ADDED TO THE CELLS WHILE BEING AGITATED. FOLLOWING A 20-MINUTE INCUBATION, THE CELLS WERE CENTRIFUGED, THE SUPERNATANT DECANTED, AND THE CELLS FIXED IN A METHANOL:GLACIAL ACETIC ACID (3:1) FIXATIVE. THE FIXATIVE WAS REMOVED AFTER CENTRIFUGATION AND REPLACED WITH FRESH FIXATIVE. THE CELLS WERE THEN REFRIGERATED OVERNIGHT. THE NEXT MORNING

THE FIXATIVE WAS CHANGED AND CELL SUSPENSIONS DROPPED ONTO CLEAN SLIDES. SLIDES WERE STAINED IN A BATH OF GIEMSA R66 (GURR) DILUTED WITH DISTILLED WATER, RINSED WITH DISTILLED WATER, DEHYDRATED IN ALCOHOL, CLEARED IN XYLENE AND MOUNTED IN DEPEX.

SLIDES WERE EXAMINED AT 1000 X MAGNIFICATION. APPROXIMATELY 50 CHROMOSOMES WERE EXAMINED FOR EACH RAT FOR A TOTAL OF 500 PER GROUP. SLIDES WERE PREPARED AT 6, 24, AND 48 HOURS FOLLOWING THE SINGLE-DOSE EXPOSURE, AND AT 6 HOURS FOLLOWING THE MUTIPLE-DOSE EXPOSURES. ABNORMALITIES RECORDED INCLUDED CHROMOSOMAL GAPS, BREAKS, FRAGMENTS, DICENTRICS, TRANSLOCATIONS AND PULVERIZATION.

RESULTS WERE COMPARED WITH POSITIVE CONTROL VALUES (ETHYL METHANE SULPHONATE--100 MG/KG DOSED ORALLY FOR 5 CONSECUTIVE DAYS FOR THE MULTIPLE DOSING PHASE AND, 250 MG/KG ONCE ONLY FOR THE SINGLE DOSING PHASE) AND WITH NEGATIVE CONTROL VALUES (AIR ONLY IN INHALATION CHAMBERS--7 HOURS/DAY FOR 5 CONSECUTIVE DAYS).

SUMMARIZED CHEMICALS, TESTS, AND RESULTS

CHEMICAL	TEST				
	DL	SM	DRL	UDS	BMC
BIS(2-METHOXYETHYL) ETHER	+	+	+?	-	+?
BUTYLENE OXIDE	-	-	-	-	-?
3-CHLOROPROPENE	-	-	-	-	-
CYCLOHEXANONE	-	-	-	-	-
HEXACHLORO BUTADIENE	+?	-	-	-	-?
2-METHOXY ETHANOL	+	+	+	-	-
METHYL BROMIDE	-	-	-	-	-
N,N-DIMETHYL ACETAMIDE	-	-	-	-	-
N,N-DIMETHYL FORMAMIDE	-	-	-	-	-
N-METHYL DICYCLOHEXYLAMINE	-	-	-	-	-
2-NITROPROPANE	-	-	-	-	-
STYRENE OXIDE	+	+	-	-	-
1,1,2,2-TETRACHLOROETHANE	*	+?	*	-	+?

\+ DENOTES A POSITIVE EFFECT

\- NO EFFECT

+? DENOTES A POSITIVE EFFECT WHOSE MEANING IS UNCLEAR

-? DENOTES A PROBABLE NO EFFECT EVEN THOUGH SOME ACTIVITY WAS RECORDED

* DENOTES THAT AN INSUFFICIENT CHALLENGE DOSE WAS ADMINISTERED

RESULTS

1. 3-CHLOROPROPENE (ALLYL CHLORIDE)

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 9,900 µG/ML OF MEDIUM

BMC, DL & SM - 1 PPM AND 25 PPM

DRL - 150 PPM FOR 7 HOURS

THERE WAS NO EVIDENCE THAT 3-CHLOROPROPENE INDUCED GENETIC DAMAGE.

2. CYCLOHEXANONE

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 9.48 MG/ML OF MEDIUM

BMC, DL & SM - 50 PPM AND 400 PPM

DRL - 50 PPM FOR 7 HOURS AND 400 PPM FOR 40 MIN.

THERE WAS NO EVIDENCE THAT CYCLOHEXANONE INDUCED GENETIC DAMAGE.

3. METHYL BROMIDE

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 70% IN AIR OVER MINIMAL MEDIUM

BMC, DL & SM - 20 PPM AND 70 PPM

DRL - 20 PPM AND 70 PPM FOR 5 HOURS

THERE WAS NO EVIDENCE THAT METHYL BROMIDE INDUCED GENETIC DAMAGE.

4. N,N-DIMETHYL ACETAMIDE

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 9,370 µG/ML OF MEDIUM

BMC, DL & SM - 20 PPM AND 700 PPM 7 HRS/DAY FOR 5 CONSECUTIVE DAYS

DRL - 200 PPM FOR 95 MINUTES

THERE WAS NO EVIDENCE THAT N,N-DIMETHYL ACETAMIDE INDUCED GENETIC DAMAGE.

5. N,N-DIMETHYL FORMAMIDE

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 9,220 µG/ML OF MEDIUM

BMC, DL & SM - 10 PPM AND 400 PPM

DRL - 400 PPM FOR 2 HOURS 15 MINUTES

THERE WAS NO EVIDENCE THAT N,N-DIMETHYL FORMAMIDE INDUCED GENETIC DAMAGE.

6. N-METHYL DICYCLOHEXYLAMINE

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 912 µG/ML OF MEDIUM

BMC, DL & SM - 5 PPM AND 25 PPM

DRL - 6 PPM FOR 4.5 MINUTES

THERE WAS NO EVIDENCE THAT N-METHYL DICYCLOHEXYLAMINE INDUCED GENETIC DAMAGE.

7. 2-NITROPROPANE

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 5,000 µG/ML OF MEDIUM

BMC, DL & SM - 25 PPM AND 200 PPM

DRL - 700 PPM FOR 4.5 HOURS

THERE WAS NO EVIDENCE THAT 3-CHLOROPROPENE INDUCED GENETIC DAMAGE.

8. BUTYLENE OXIDE

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 7,300 µG/ML OF MEDIUM

BMC, DL & SM - 250 PPM AND 1000 PPM

DRL - 1000 PPM FOR 7 HOURS

ALL ASSAYS WERE NEGATIVE WITH THE EXCEPTION OF THE BMC TEST. HOWEVER, AN INCREASE IN THE FREQUENCY OF ABERRANT CELLS WAS FOUND IN ONLY ONE SAMPLE GROUP (MALES--SINGLE 1000 PPM EXPOSURE--SAMPLED AT 6 HOURS), AND THAT EFFECT WAS SMALL AND DID NOT OCCUR IN FEMALES. IT WAS CONCLUDED THAT THERE WAS NO EVIDENCE THAT BUTYLENE OXIDE IS CAPABLE OF INDUCING GENETIC DAMAGE.

9. BIS(2-METHOXYETHYL) ETHER

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 19,000 µG/ML OF MEDIUM

BMC, DL & SM - 250 PPM AND 1000 PPM

DRL - 250 PPM FOR 2.75 HOURS

THE UDS ASSAY REVEALED NO GENETIC DAMAGE.

THE BMC ASSAY REVEALED SIGNIFICANT DECREASES IN ABERRANT CELL FREQUENCIES IN THREE EXPOSURE GROUPS; MULTIPLE DOSING AT 1000 PPM AND THE SINGLE DOSING (48 HR READING) AT 250 PPM AND 1000 PPM; WHILE A FOURTH GROUP, SINGLE DOSING (6 HR READING) AT 250 PPM SHOWED A SIGNIFICANT INCREASE IN ABERRATIONS. AN INAPPROPRIATE CONTROL RESPONSE, I.E., NUMEROUS ABERRATIONS, PROBABLY ACCOUNTS FOR THE DECREASE IN THE TREATED GROUPS. THE INCREASE IN THE 250 PPM TREATMENT GROUP WAS PROBABLY SPURIOUS SINCE IT DID NOT OCCUR IN THE 1000 PPM GROUP.

THE DRL TEST YIELDED RESULTS THAT MAY BE SUGGESTIVE OF A GENETIC EFFECT, ALTHOUGH THEY WERE NOT ENTIRELY CLEAR AND REMAIN UNCONFIRMED. A SINGLE BROOD SHOWED A SMALL BUT SIGNIFICANT INCREASE ($P < 0.05$) OVER THE CORRESPONDING CONTROL VALUE. THIS EFFECT WAS NOT SEEN IN OTHER BROODS NOR IN ANOTHER STOCK. WHEN POOLED WITH THE OTHER CONCURRENT BROODS, THIS EFFECT WAS NOT SIGNIFICANT.

FOR THE SM TEST, THE ACCOMPANYING GRAPH (FIGURE 1) DEMONSTRATES A CLEAR INCREASE IN ABNORMALITIES. THE GREATEST INCREASE WAS IN SPERM WITH AMORPHOUS HEADS.

AS SEEN IN TWO ADDITIONAL GRAPHS (FIGURES 2 AND 3), THE DL ASSAY DEMONSTRATED A SIGNIFICANT ANTI-FERTILITY EFFECT. MOST SIGNIFICANTLY, THE NUMBER OF IMPLANTATIONS WAS GREATLY REDUCED IN FEMALES MATED TO MALES ON WEEKS SIX AND SEVEN POST-EXPOSURE. ALSO, THE PERCENT OF FEMALES THAT WERE PREGNANT WAS GREATLY REDUCED WHEN MATED TO MALES ON WEEKS FIVE THROUGH SEVEN POST-EXPOSURE. THERE WAS ALSO A SLIGHT INCREASE IN THE FREQUENCY OF EARLY DEATHS IN BOTH EXPOSURE GROUPS.

10. HEXACHLORO-1,3,-BUTADIENE

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 1 MG/ML OF MEDIUM

BMC, DL & SM - 10 PPM AND 50 PPM

DRL - 25 PPM FOR 1 HOUR

THE UDS, DRL, AND SM ASSAYS WERE NEGATIVE.

IN THE BMC TEST, ALTHOUGH A SMALL BUT SIGNIFICANT INCREASE IN ABERRATIONS WAS NOTED IN THE 10 PPM EXPOSURE GROUP, ONLY 2 WERE SEEN IN THE 50 PPM GROUP; THEREFORE, IT IS CONCLUDED THAT HEXACHLORO-1,3-BUTADIENE HAD NO EFFECT UPON THE FREQUENCY OF ABERRANT CELLS.

IN THE DL ASSAY, A SMALL BUT STATISTICALLY SIGNIFICANT REDUCTION ($P < 0.01$) IN IMPLANTATIONS WAS NOTED IN ONE GROUP OF ANIMALS, WEEK 1 OF THE 50 PPM GROUP. IT IS NOT CLEAR THAT THIS WAS TREATMENT RELATED.

11. 2-METHOXY ETHANOL

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 9,660 µG/ML OF MEDIUM

BMC, DL & SM - 25 PPM AND 500 PPM

DRL - 500 PPM FOR 15 MINUTES AND 25 PPM FOR 1 HOUR

THE UDS AND BMC ASSAYS WERE NEGATIVE.

SEVERAL DRL TESTS WERE PERFORMED AND RESULTS INDICATED A LOW LEVEL A GENOTOXIC ACTIVITY.

THE ACCOMPANYING GRAPH (FIGURE 4) DEMONSTRATES AN INCREASE SEEN IN THE FREQUENCY OF SPERM ABERRATION IN THE 500 PPM EXPOSURE GROUP. THE 25 PPM GROUP WAS UNAFFECTED.

FOR THE DL ASSAY, TWO ADDITIONAL GRAPHS (FIGURES 5 AND 6) DEMONSTRATE THE PROFOUND EFFECT ON MALE FERTILITY IN THE 500 PPM EXPOSURE GROUP, ALTHOUGH RECOVERY DID OCCUR. THE PERIOD ENCOMPASSING THE FIFTH THROUGH SEVENTH WEEKS FOLLOWING MALE EXPOSURE WAS THE MOST SIGNIFICANT FOR PRODUCING BOTH THE GREATLY REDUCED NUMBER OF IMPLANTS PER PREGNANCY, AND IN CAUSING THE DRASTIC REDUCTION IN THE PERCENT OF FEMALES THAT WERE IMPREGNATED.

12. STYRENE OXIDE

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 50 µG/ML OF MEDIUM

BMC, DL & SM - 15 PPM AND 100 PPM

DRL - 100 PPM FOR 2.5 HOURS

THE UDS, DRL, AND BMC ASSAYS WERE NEGATIVE.

AT THE HIGHER DOSE LEVEL (100 PPM), THE SM ASSAY SHOWED A SMALL INCREASE ($P < 0.05$) IN THE FREQUENCY OF ABERRANT SPERM IN ONE CATEGORY (AMORPHOUS HEADS).

THE DL TEST SHOWED NO INCREASE IN THE FREQUENCY OF EARLY DEATHS. THE FREQUENCY OF PREGNANCY WAS NOT SIGNIFICANTLY REDUCED. A SLIGHT ANTI-FERTILITY EFFECT WAS SEEN AS A REDUCTION IN THE TOTAL NUMBER OF IMPLANTATIONS PER PREGNANCY. HOWEVER, THIS OCCURRED ONLY AT THE HIGHER CONCENTRATION (100 PPM) AND ONLY IN FEMALES MATED AT THE ONE WEEK POST-EXPOSURE PERIOD.

13. 1,1,2,2,-TETRACHLOROETHANE

ASSAYS - EXPOSURES: UDS - 3-HOUR EXPOSURES USING A SERIES OF 8 CONCENTRATIONS UP TO 9,900 µG/ML OF MEDIUM

BMC, DL & SM - 5 PPM AND 50 PPM

DRL - 5 PPM FOR 7 HOURS AND 50 PPM FOR 40 MINUTES

THE UDS, DRL, AND DL ASSAYS WERE NEGATIVE. HOWEVER, IN THE DRL AND DL TESTS, CONCENTRATIONS WERE USED THAT DID NOT EXTEND INTO THE TOXIC RANGE AND, THEREFORE, 1,1,2,2-TETRACHLOROETHANE HAS NOT BEEN TESTED TO THE PRACTICAL LIMITS OF THESE TWO ASSAYS.

THE BMC ASSAY SHOWED A SMALL INCREASE ($P < 0.05$) IN ABERRANT CELL FREQUENCY IN A SINGLE EXPOSURE GROUP. THIS DIFFERENCE WAS SEEN ONLY WHEN CHROMOSOMAL GAPS WERE EXCLUDED FROM THE COMPARISON AND WAS SEEN ONLY IN THE 50 PPM SINGLE EXPOSURE (6-HR SAMPLING), FEMALE RAT GROUP. ALL MALES, ALL OTHER FEMALES, AND ALL THE MULTIPLE-EXPOSURE GROUPS WERE UNAFFECTED.

THE SM ASSAY REVEALED A SMALL INCREASE ($P < 0.05$) IN THE 50 PPM EXPOSURE GROUP OF SPERM WITH HOOK ABNORMALITIES, HOWEVER, ANY BIOLOGICAL SIGNIFICANCE OF SUCH AN INCREASE IS DOUBTFUL.

NONE OF THESE RESULTS DEMONSTRATED CONVINCING EVIDENCE OF GENOTOXICITY.

FIGURE 1

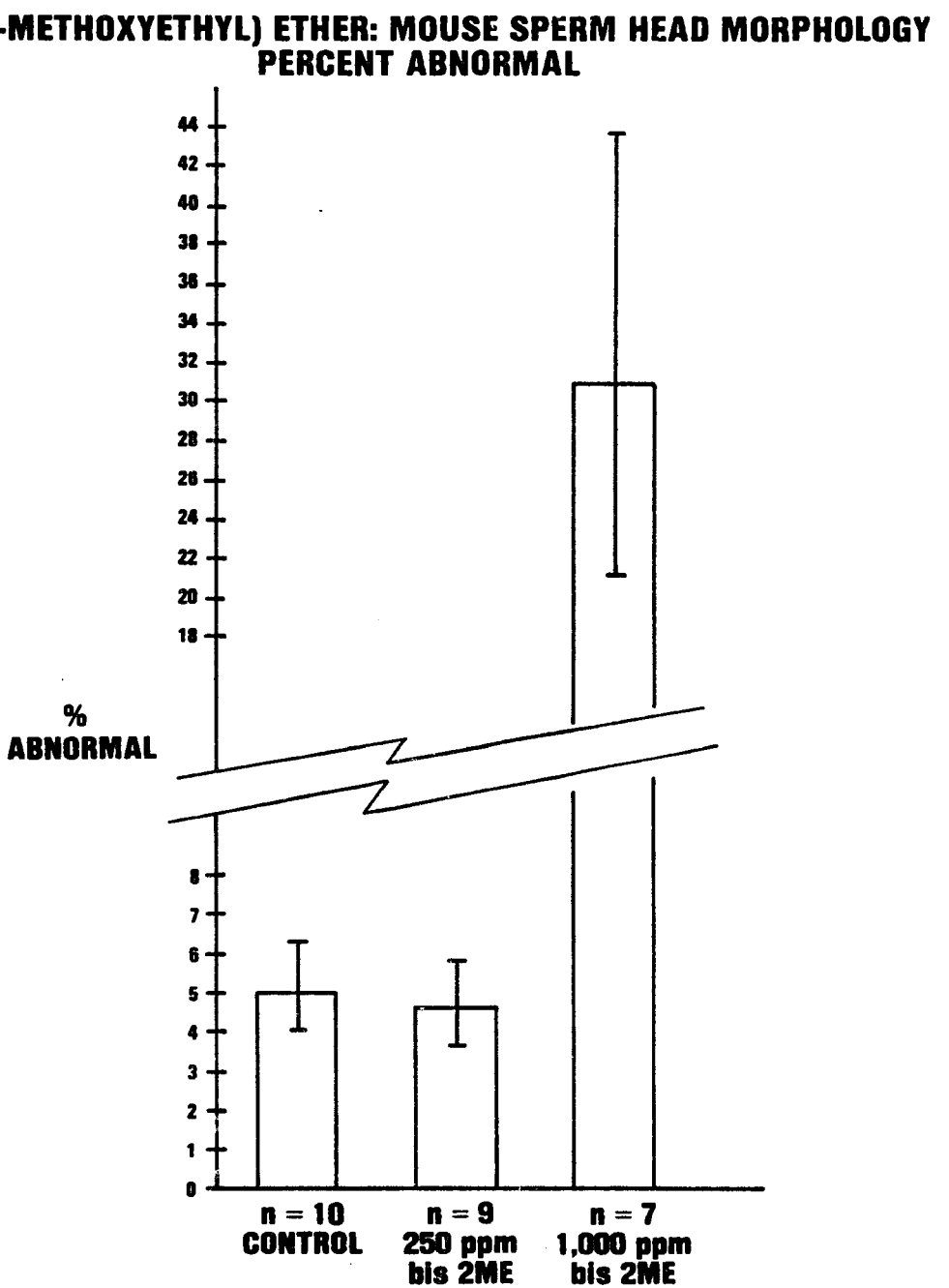

bis (2-METHOXYETHYL) ETHER: MOUSE SPERM HEAD MORPHOLOGY
PERCENT ABNORMAL
%
ABNORMAL
44
42
40
38
36
34
32
30
28
26
24
22
20
18
8
7
6
5
4
3
2
1
0
n = 10
CONTROL
n = 9
250 ppm
bis 2ME
n = 7
1,000 ppm
bis 2ME

FIGURE 2

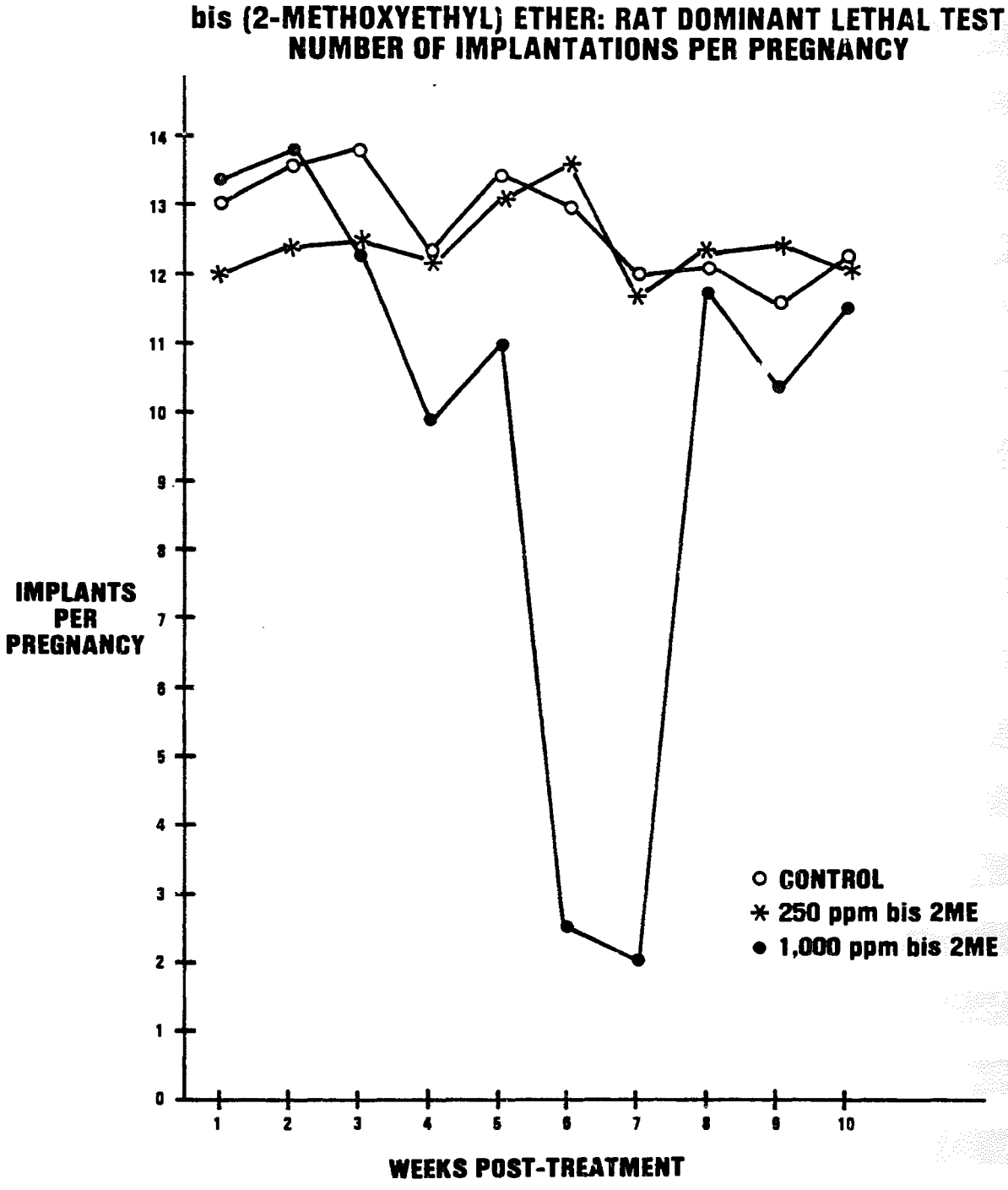

bis (2-METHOXYETHYL) ETHER: RAT DOMINANT LETHAL TEST
NUMBER OF IMPLANTATIONS PER PREGNANCY
IMPLANTS PER PREGNANCY
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
1
2
3
4
5
6
7
8
9
10
WEEKS POST-TREATMENT
CONTROL
250 ppm bis 2ME
1,000 ppm bis 2ME

FIGURE 3

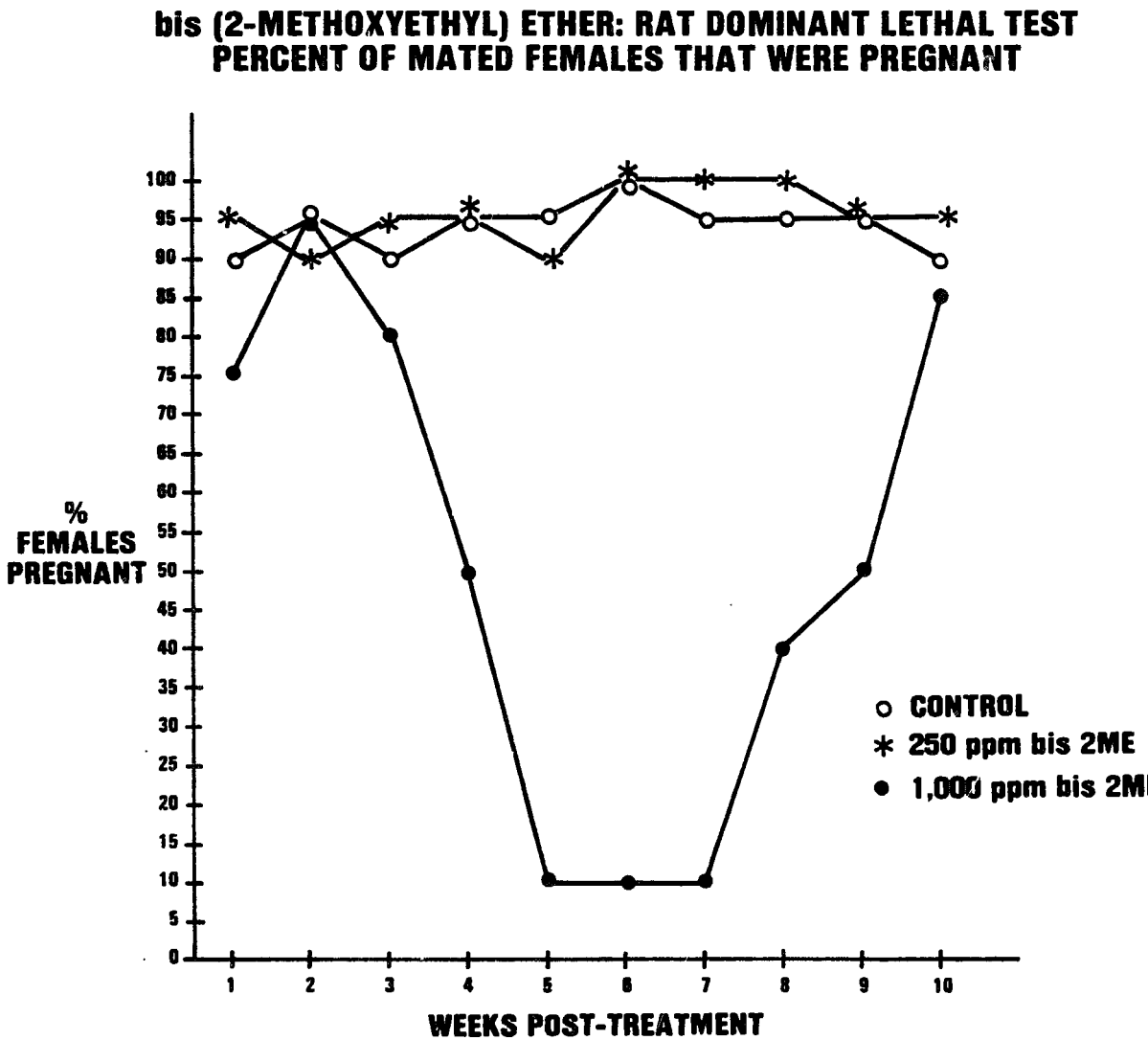

bis (2-METHOXYETHYL) ETHER: RAT DOMINANT LETHAL TEST
PERCENT OF MATED FEMALES THAT WERE PREGNANT
% FEMALES PREGNANT
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
WEEKS POST-TREATMENT
CONTROL
250 ppm bis 2ME
1,000 ppm bis 2ME

FIGURE 4

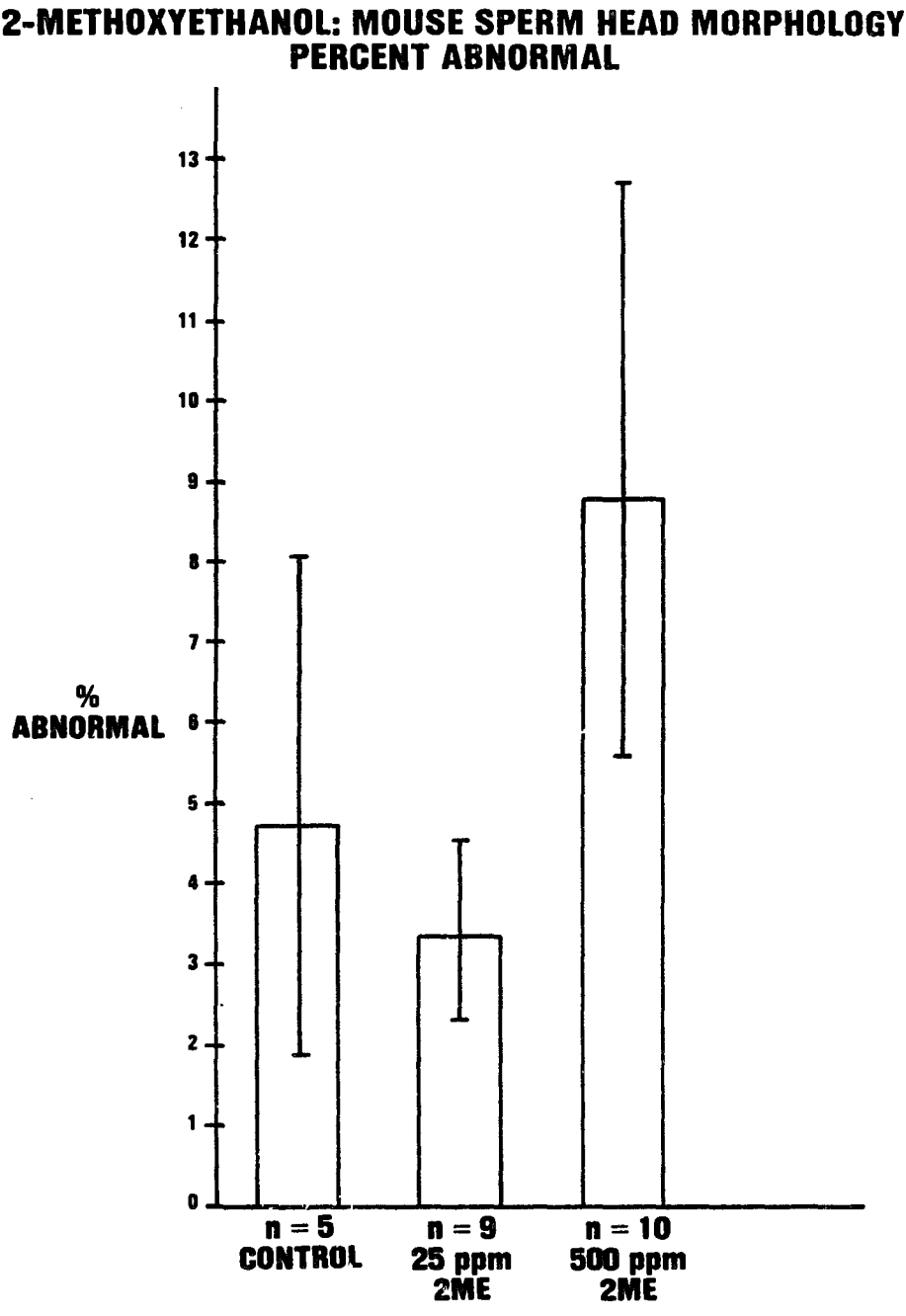

2-METHOXYETHANOL: MOUSE SPERM HEAD MORPHOLOGY
PERCENT ABNORMAL
%
ABNORMAL
0
1
2
3
4
5
6
7
8
9
10
11
12
13
n = 5
CONTROL
n = 9
25 ppm
2ME
n = 10
500 ppm
2ME

FIGURE 5

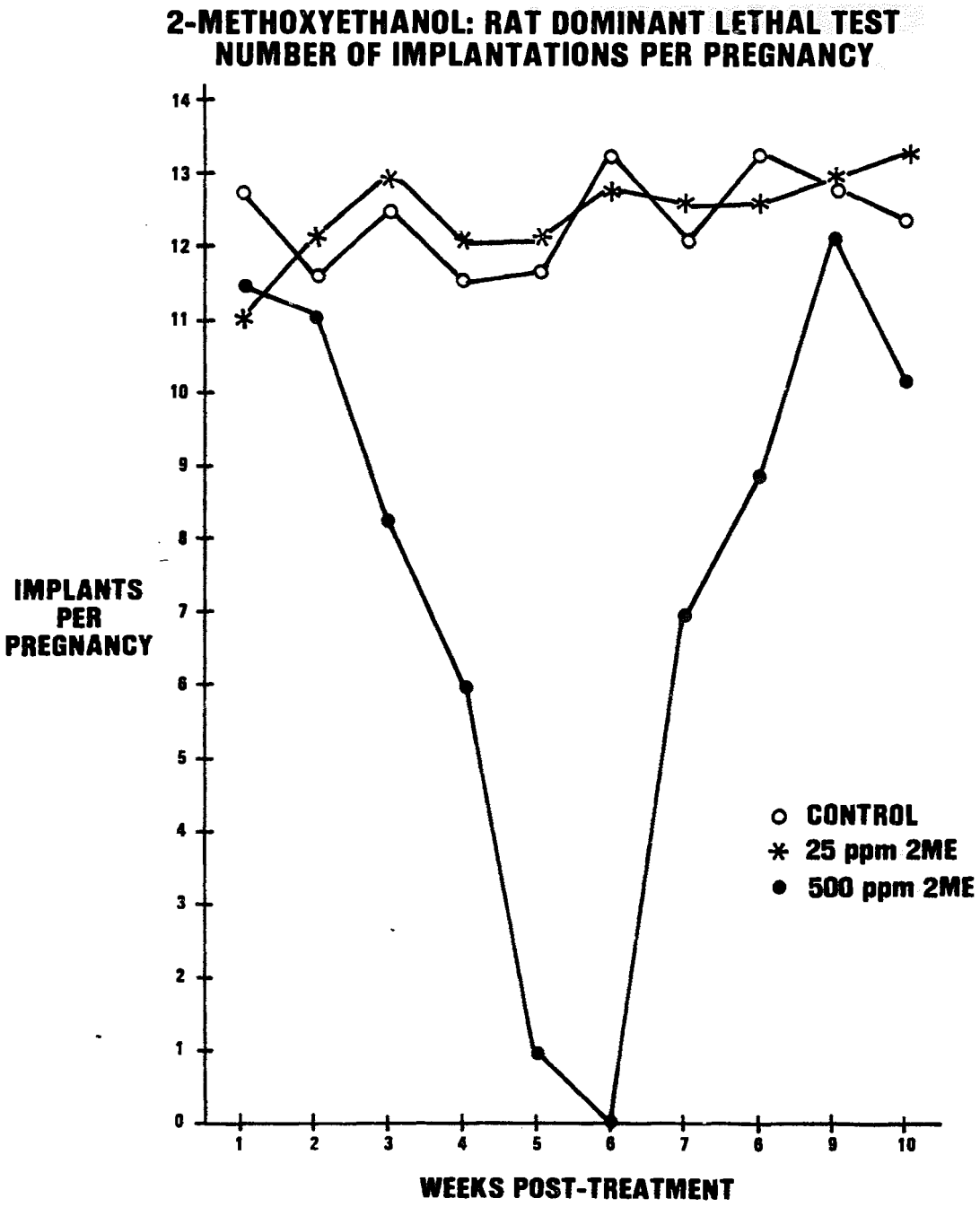
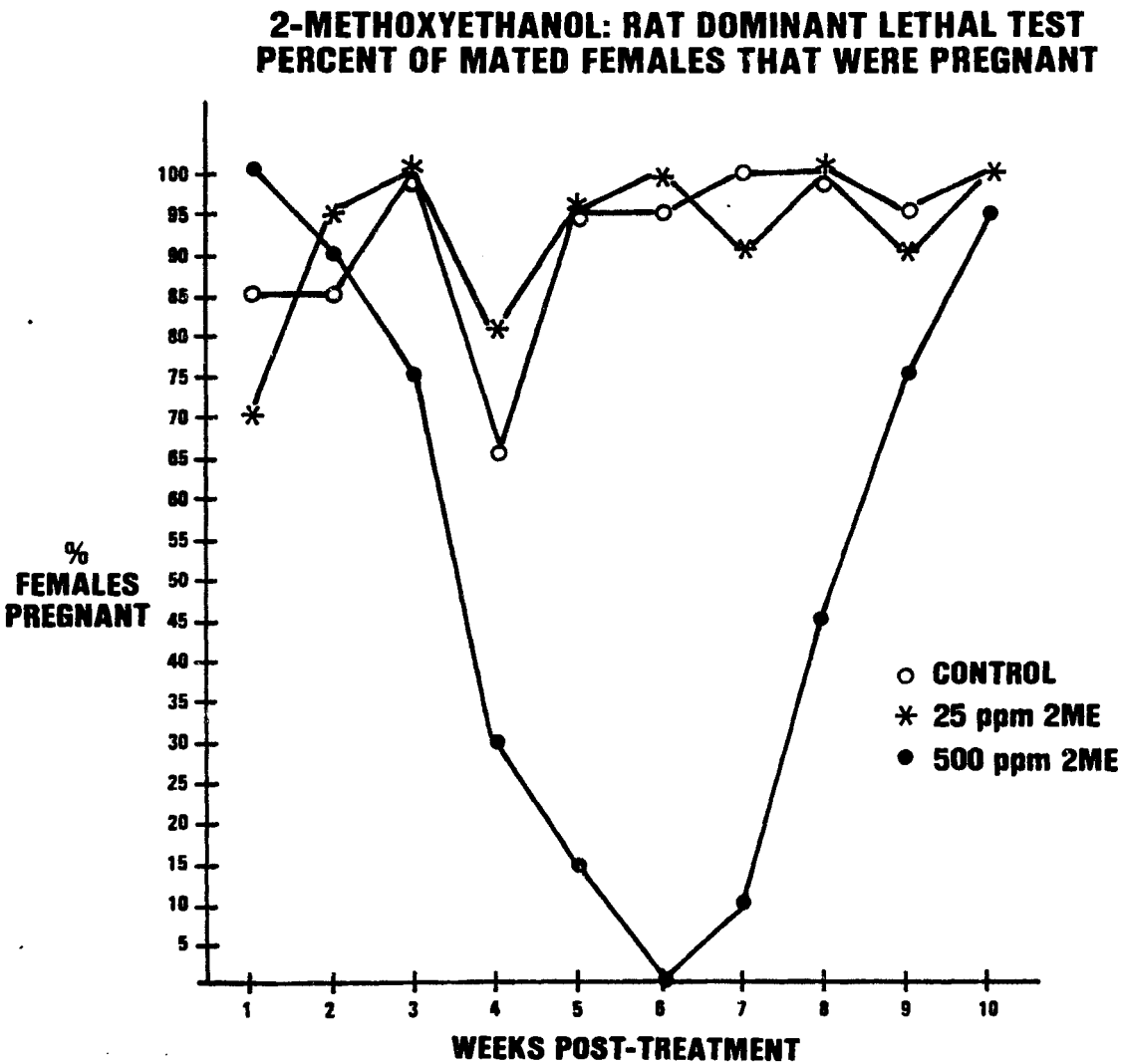

2-METHOXYETHANOL: RAT DOMINANT LETHAL TEST
NUMBER OF IMPLANTATIONS PER PREGNANCY
IMPLANTS PER PREGNANCY
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
1
2
3
4
5
6
7
8
9
10
WEEKS POST-TREATMENT
CONTROL
25 ppm 2ME
500 ppm 2ME

FIGURE 6


2-METHOXYETHANOL: RAT DOMINANT LETHAL TEST
PERCENT OF MATED FEMALES THAT WERE PREGNANT
% FEMALES PREGNANT
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
1
2
3
4
5
6
7
8
9
10
WEEKS POST-TREATMENT
CONTROL
25 ppm 2ME
500 ppm 2ME