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SECURITY CLASSIFICATION-DE SÉCURITÉ

OUR FILE - NOTRE RÉFÉRENCE

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DATE Feb. 13, 1980

Validation of Teratologic Investigation of Phaltan (FOLPET) in Macaca mulatta (Rhesus Monkey) and Macaca arctoides (Stumptailed Macague)(IBT No. M5519).

Overall Comments:

This report was produced from microfiche provided for a Difolatan study which was conduced under the same IBT number as phaltan and captan and at approximately the same time. Considerable raw data are missing on daily breeding records for the stumptailed macaques and for most of the animals given thalidomide. Although the final report indicated that there were no negative controls, the correspondence indicated that FDA wanted controls to be obtained concurrently. Since there was such a low fertility rate in the 3 phaltan-treated groups, (only 26 fetuses from 173 animals mated and dosed), it is difficult, without negative controls, to know if the low fecundity was the result of breeding difficulties in this particular colony or caused by the test material. The use of such large numbers of matings to achieve the resultant fetuses was not mentioned in the final report. It was also apparent that different test materials or different amounts of the same test material, were in many cases, used in each of several successive breeding cycles in the same animal. Considering all the above mentioned facts, this study should be considered to be invalid.

Teratologic Investigation of Phaltan in Macaca mulatta (Rhesus Monkey) and Macaca arctoides (Stumptailed Macague) (IBT No. M5519).

A. Audit:

1. Report No: IBT No. M5519, dated Dec. 1, 1969.

2. Date of Study: Started on 5-9-68 and terminated on 26-11-69.

3. Protocol: A protocol was available (MF-100) which indicated that sexually mature female Rhesus monkeys (it was later decided with FDA permission to use an approximately equal number of Stumptailed macagues) would be mated based on perineal turgesence, vaginal smears and interpretation of previous cycles. Females were to be placed with males for 2 days for mating (10th to 12th day after menstration began). The 11th day was considered to be Day 1 of pregnancy. Ten females were dosed daily from the 18th through the 31st day of pregnancy (14 doses) at each of 3 dose levels: 10, 25 and 75 mg/kg. (given orally by gelatin capsule). An additional 10 females were to have received 10 mg of thalidomide/kg of body weight from day 23 to day 25 of gestation (the final report indicated that 11 rhesus and 6 stumptails received 10 mg/kg of thalidomide and 3 stumptails received 5 mg/kg of thalidomide/day). Twenty control females were to have received empty capsules (no controls were used). All animals were to be weighed prior to mating and weeky thereafter. The fetus was to be removed from each animal on the 10th week of gestation, weighed, examined for external and skeletal (X-ray) malformations, and fetal membranes were to be examined and diameter recorded.

4. Test Material: Phaltan technical (1 lb) was shipped to IBT, on 30-8-67 and again on 15-11-67.

5. Animal Suitability: Rhesus monkeys and stumptailed macagues were used. No invoices for their purchase were found in the data package.

6. Raw Data: No daily observations were available on any

of the stumptailed macaques and no dosage sheets were available on any of the stumptailed macaques used at the 75 mg/kg level of Phaltan or thalidomide. No dosage sheets were available for 7 of the 11 Rhesus monkeys acutally given thalidomide. Pathology and organ weight data were available for only 1 of the monkeys given thalidomide.

B. Validation:

1. Dates: The first monkey (No. 68-82) was dosed on 5-9-68 (MF-1120) and a fetus was delivered from the last monkey (R-79) on 26-11-69 (MF-2032).

2. Protocol: According to the "Procedure", the following changes were made in the above protocol.

1. 25 female rhesus monkeys and 22 stumptails (4 of each per dose level), were used with no controls while the original protocol called for 10 rhesus monkeys at each dose level. The use of stumptails was approved by FDA but correspondence (no numbers on microfiche) indicated that Dr. Blumethal only approved of it if adequate control data were obtained and presented concurrently.

2. Males were left with females for 7 days instead of 2. This change appeared to have been made by IBT (see internal memo from J.F.V. - MF-65 on 7-4-69).

3. The procedure indicates that the females were weighed at the beginning and midpoint of the dosing period while the protocol calls for weekly weighing. The person responsible for making this change was not indicated

4. The procedure indicates that phaltan and thalidomide were often mixed in coconut cream and fed rather than by gelatin capsule as specified in the protocol. The raw data do not indicate who authorized this change.

5. The procedure calls for weighing fetal organs (brain, lung, liver, kidney, heart and spleen) and placenta but this was not specified in the protocol. Again, no authorization for this change was found.

The study appears to have been performed more in line with the procedure than with the protocol but with some raw data missing it is difficult to determine exactly how closely it was followed.

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3. Test Material & Quality Control: Invoices indicate that enough of the test material to perform the study (two 1 lb lots) were shipped to IBT in time for the study.

4. Personnel: Report prepared by: J. F. Vondruska, D.V.M.
Section Head
Primate & Dog Toxicity

Report approved by: O. E. Fancher, Ph.D.
Director.

5. Execution of the study:

a. Body weight: Body weights of females were determined on the day they were first dosed and 1 week later according to the procedure but these weights were not given in the final report.

b. Dosage of test material and thalidomide: Raw data on the dosage of each individual monkey according to body weight were handwritten on individual dosage sheets for each pregnancy. They were dated and signed and agreed with the final report except for the 4 stumptailed macagues at 75 mg/kg and 16 of the 20 animals given thalidomide for which no dosing sheets were available. Evidence in the microfiche (MF-51) indicates that at least 5 of the rhesus monkeys given thalidomide belonged to a study done earlier by Bionetics.

c. Breeding Observations: Handwritten, and dated daily observations on each monkey indicate that at least 67 animals were dosed at 10 mg/kg/day for 1 to 14 days in each of 78 pregnancies; 22 animals were dosed at 25 mg/kg/day for 1 to 14 days in each of 30 pregnancies; and 48 animals were dosed at 75 mg/kg/day for 1 to 14 days in each of 65 pregnancies (all dosages were phaltan). Quite a number of these animals were dosed at more than one level of phaltan and this was sometimes preceded with dosing in earlier cycles with captan or difolatan. Dosage with thalidomide sometimes followed phaltan in later pregnancies but in only 1 of the 20 animals reported to have been dosed with thalidomide (No. R-208) was dosage and production of a fetus confirmed by the raw data.

One stumptail (S-40) at 10 mg/kg/day was only dosed for 7 days beginning on 18-7-69 (MF 1264) but was credited with producing a fetus by caesarian on 19-9-69. Since no daily observations (including size of uterus by palpation, confirmation of dosage, menses, etc) were available for any of the 13 stumptails given phaltan in this study, it was not possible to know if S-40 was really pregnant or the fetus sent to pathology came from another animal. In all the earlier observations sheets, dosage was only stopped before 14 days if the animal was not pregnant.

Since pregnancy could not be confirmed by rectal palpation until the animal had been dosed for several days it was impossible to know if that animal really was not pregnant or if the phaltan caused resorption or abortion. Occasionally an observation of "probably pregnant" uterus 1-2 cm" was made (e.g. MF 2845) during the dosing period. The animal was later found to be "not pregnant". It was possible but not certain that the test material may have been responsible for the termination of pregnancy. Only if the uterus exceeded 2 cm in diameter was pregnancy more of a certainty and in no case with phaltan was this found to happen without the pregnancy continuing to a reported abortion (1 case) or caesarian. Since no negative controls were used in this study, counter to the original protocol and suggestions of FDA, it is difficult to know if the low fertility rate in the colony was real or caused by the test material.

d. Organ Weights of Fetuses: Handwritten and dated raw data on fetal weights and organ weights were available on pathology sheets and agreed with the report for all fetuses in the phaltan-treated groups and for 1/20 fetuses given thalidomide except for a few minor errors in number of days of gestation and the following errors: placenta of fetus S-30-F (10 mg/kg) was 35.06 instead of 45.06 g; the lung/body wt ratio of fetus S-49-F (25 mg/kg) was 36.3 instead of 31.4; and the heart weight and heart/body ratio of fetus S-41-M were 2.33 instead of 0.23 and 36.97 instead of 3.20, respectively. Whether or not this heart was actually enlarged as indicated is not known.

e. Pathology: The raw data were handwritten on dated pathology sheets. Although the raw data did not support the final report in showing that 2 animals, 1 at 25 mg/kg and 1 at 75 mg/kg, had 13 ribs, it did show that one animal at 75 mg/kg (No. S-36-F) (MF-3022) had only 1 ovary (on left side), one fetus (25 mg/kg) (S-47-F)(MF-3026) had "internal hemmorage around intestines" and No. R-202-F had "HEM on liver" and Cong. on lungs". Gross pathology was available on only 1 of the fetuses (R-208-M) from the thalidomide groups (10 mg/kg) and the raw data agreed with that in the final report except it failed to mention "deformed clavicle on L arm" and "deformed L kidney". The pathology sheets indicate 4 normal fetuses were delivered by caesarian during the last few days of the study from stumptails treated with thalidomide (10 mg/kg) (No.s S-52, S-68, S-80 and S-107) that were not reported (see MF-3027, 3030, 3032 and 3035, respectively).

Overall Comments: This report was produced from microfiche provided for a Difolatan study which was conducted under the same IBT number as Phaltan and at approximately the same time. Considerable raw data are missing on daily breeding records for the stumptailed macaques and for most of the animals given thalidomide. Although the final report indicated that there were no negative controls, the correspondence indicated that FDA wanted controls to be obtained concurrently. Since there was such a low fertility rate in the 3 phaltan-treated groups, (only 26 fetuses from 172 animals mated and dosed), it is difficult, without negative controls, to know if the low fecundity was the result of breeding difficulties in this particular colony or caused by the test material. The use of such large numbers of matings to achieve the resultant fetuses was not mentioned in the final report. Considering all the above mentioned facts, this study should be considered to be invalid.

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