

5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY

| | |
|--------------------------|---|
| Species/strain: | Sprague-Dawley Cri:CD® (SD) IGS BR strain |
| Sex/Number: | 96 time-mated female rats |
| Age: | 10-12 weeks |
| Route of Administration: | Oral gavage |
| Duration of the test: | All animals were killed by carbon dioxide asphyxiation followed by cervical dislocation on Day 20 of gestation. |
| Exposure period: | Animals were dosed on Day 5 to Day 19 of gestation. |
| Doses: | 100, 300 and 1000 mg/kg/day |
| Control group: | Yes (Concurrent vehicle – arachis oil 4 ml/kg/day) |
| NOEL Maternal Toxicity: | 1000 mg/kg/day |
| NOEL teratogenicity : | 1000 mg/kg/day |

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Results:

Overview

Mortality. There were no unscheduled deaths.

Clinical Observations. No clinically observable signs of toxicity were detected for test or control animals throughout the study period.

Bodyweight. No adverse effect on bodyweight development was detected.

Food Consumption. No adverse effect on dietary intake was detected.

Post Mortem Studies. No macroscopic abnormalities were detected in females at terminal kill. No treatment-related effects were detected in the uterine parameters examined, in fetal viability or in growth and development.

Fetal Evaluation. No treatment-related effects were detected on skeletal development or in the type and incidence of skeletal or visceral findings. (Tables 1-3).

Conclusion. The oral administration of IRGANOX 3114 to pregnant rats by gavage during organogenesis at dose levels of 100, 300, 1000 mg/kg/day produced no toxicologically significant changes in the adult or offspring parameters measured. The No Observed Effect Level (NOEL) was, therefore, considered to be 1000 mg/kg/day.

Discussion

Females from all treatment groups showed a statistically significant increase in the number of fetuses showing seven or more ossified post lumbar vertebral centra. Consequently the number of fetuses showing less than seven ossified post lumbar vertebral centra was lower for all treatment groups. The expected number of ossified post lumbar vertebral centra is seven thus indicating a higher incidence of fetuses with expected or above expected degrees of ossification. The dose-related response was unconvincing and all group mean percentages were within historical ranges for rats of the strain and age used; as such intergroup difference was considered to be without toxicological importance.

Females from all treatment groups showed a statistically significant reduction in the number of fetuses showing two or

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1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6 (1H,3H,5H)-trione

more ossified phalanges and consequently an increase in the number of fetuses showing less than two ossified phalanges. Ossification of phalanges in Day 20 fetuses is regarded as precocious ossification and therefore represents an unexpected degree of foetal development. It is considered that a treatment-related effect on precocious ossification is not representative of a developmental toxic event as there is no concomitant effect upon what is regarded as normal ossification (or other limb bones). The dose-related response was unconvincing and since it was control values which had the higher incidence of precocious ossification the intergroup differences were not considered evidence of a treatment-related effect.

Females treated with 1000 and 300 mg/kg/day showed a statistically significant increase in the number of fetuses showing greater than six ossified metatarsals. The standard number of ossified metatarsals in Day 20 fetuses is greater than six, therefore, the intergroup differences indicate a higher number of fetuses showing a normal number of ossified metatarsals compared to control and as such, is considered of no toxicological importance.

Females from all treatment groups showed a statistically significant increase in the number of fetuses showing a medium fontanelle, and subsequently a reduction in the number of fetuses showing a small fontanelle. The standard classification of fontanelle size is medium and therefore the intergroup differences were considered to be attributable to the increased number of control fetuses showing a small fontanelle, of which the values were outside historical ranges for rats of the strain and age used, and as such, were considered of no toxicological significance.

| | |
|-------------------|---|
| Method: | OECD 414 |
| GLP: | Yes |
| Test substance: | 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione. Batch number 10139JW4. |
| Dose Analysis: | Chemical analysis demonstrated that dose formulations were within $\pm 4\%$ of nominal |
| Remarks: | This study was assigned a reliability code of 1a ² (guideline study) according to the criteria established by Klimisch <i>et al</i> (1997). |
| Reference: | Irganox 3114: Oral Gavage Prenatal Development Toxicity Study in the Rat. SPL PROJECT NUMBER: 1976/002. SafePharm Laboratories, Derbyshire, UK. 18 November 2005. |

²Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997

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Table 1. Group Incidence of Fetal Visceral Findings

| | Dose Level (mg/kg/day) | | | | | | | | | | | |
|----------------------------------|----------------------------|----|------|-----|----|------|-----|----|------|------|----|------|
| | 0 (Control) | | | 100 | | | 300 | | | 1000 | | |
| | Number of Fetuses Examined | | | | | | | | | | | |
| | 134 | | | 131 | | | 143 | | | 151 | | |
| | NF | NL | %† | NF | NL | %† | NF | NL | %† | NF | NL | %† |
| Head | | | | | | | | | | | | |
| a | 2 | 2 | 1.4 | 1 | 1 | 0.6 | 1 | 1 | 0.6 | 2 | 1 | 1.2 |
| b | 2 | 1 | 1.7 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 0.5 |
| c | 5 | 2 | 3.3 | 3 | 2 | 2.6 | 2 | 2 | 1.3 | 2 | 2 | 1.3 |
| d | | 2 | 3.6 | 1 | 1 | 1.0 | 0 | 0 | 0.0 | 1 | 1 | 0.5 |
| e | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 1.1 | 0 | 0 | 0.0 |
| Neck/Thorac | | | | | | | | | | | | |
| f | 6 | 5 | 4.8 | 8 | 6 | 5.8 | 5 | 4 | 3.2 | 3 | 2 | 2.0 |
| g | 2 | 2 | 1.7 | 1 | 1 | 0.7 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| h | 17 | 8 | 13.6 | 20 | 8 | 14.3 | 11 | 4 | 7.3 | 22 | 10 | 15.3 |
| j | 2 | 1 | 1.7 | 2 | 2 | 1.2 | 3 | 2 | 2.4 | 1 | 1 | 0.5 |
| k | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 0.6 |
| l | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 0.7 |
| m | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 0.7 |
| n | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 2 | 2 | 1.3 |
| o | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 0.5 |
| p | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 0.5 |
| Abdomen | | | | | | | | | | | | |
| q | 1 | 1 | 0.8 | 0 | 0 | 0.0 | 1 | 1 | 0.8 | 0 | 0 | 0.0 |
| r | 0 | 0 | 0.0 | 1 | 1 | 0.7 | 3 | 3 | 2.1 | 3 | 3 | 2.0 |
| s | 3 | 2 | 2.5 | 7 | 4 | 4.3 | 5 | 4 | 3.5 | 1 | 1 | 0.7 |
| t | 16 | 8 | 12.9 | 16 | 8 | 11.7 | 9 | 4 | 6.0 | 12 | 8 | 8.6 |
| u | 8 | 4 | 6.7 | 10 | 6 | 6.6 | 9 | 4 | 6.7 | 10 | 7 | 7.2 |
| v | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 0.7 |
| w | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 0.7 |
| Total number of affected fetuses | 35 | 12 | 26.1 | 33 | 13 | 23.6 | 27 | 12 | 18.8 | 36 | 19 | 24.3 |

NF = Number of fetuses

NL = Number of litters

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Table 2 Group Incidence of Fetal Skeletal Findings

| | Dose Level (mg/kg/day) | | | | | | | | | | | |
|--|----------------------------|----|------|-----|----|------|-----|-----|------|------|----|------|
| | 0 (Control) | | | 100 | | | 300 | | | 1000 | | |
| | Number of Fetuses Examined | | | | | | | | | | | |
| | 126 | | | 121 | | | 133 | | | 139 | | |
| | NF | NL | %† | NF | NL | %† | NF | NL | %† | NF | NL | %† |
| Head/Neck | | | | | | | | | | | | |
| a Incomplete ossification of one cranial bone (variant) | 19 | 8 | 15.0 | 11 | 6 | 8.1 | 29 | 15 | 21.5 | 17 | 7 | 12.2 |
| b Incomplete ossification of more than one cranial bone (variant) | 19 | 6 | 13.9 | 23 | 9 | 18.2 | 11 | 6 | 8.2 | 13 | 5 | 9.4 |
| c Irregular ossification of one cranial bone (variant) | 5 | 5 | 3.8 | 8 | 4 | 6.3 | 9 | 5 | 6.9 | 6 | 4 | 4.1 |
| d Irregular ossification of more than one cranial bone (variant) | 4 | 3 | 3.1 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 6 | 3 | 4.2 |
| e Incomplete ossification of one facial bone (variant) | 2 | 1 | 1.3 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| f Incomplete ossification of more than one facial bone (variant) | 1 | 1 | 0.8 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| g Extra area of ossification between parietals and interparietal | 1 | 1 | 1.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| h Hyoid not ossified | 21 | 10 | 16.6 | 26 | 12 | 24.2 | 27 | 11 | 19.6 | 17 | 9 | 13.3 |
| Ribs | | | | | | | | | | | | |
| j Unilateral/bilateral rudimentary 13th ribs (variant) | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 0.6 | 0 | 0 | 0.0 |
| k Unilateral/bilateral rudimentary 14th ribs (variant) | 1 | 1 | 0.8 | 0 | 0 | 0.0 | 0 | 0.0 | 0.0 | 1 | 1 | 0.6 |
| l Unilateral/bilateral shortened 13th rib (s) (variant) | 5 | 3 | 4.0 | 1 | 1 | 0.8 | 4 | 2 | 2.7 | 5 | 5 | 3.0 |
| m More than one rib wavey | 2 | 1 | 1.7 | 1 | 1 | 1.2 | 1 | 1 | 0.8 | 0 | 0 | 0.0 |
| n One rib wavey | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0.0 | 0.0 | 2 | 1 | 1.4 |
| Vertebrae | | | | | | | | | | | | |
| o One thoracic vertebral center semi-bipartite (variant) | 15 | 10 | 12.0 | 4 | 4 | *2.9 | 16 | 10 | 12.9 | 20 | 14 | 13.3 |
| p More than one thoracic vertebral center semi-bipartite (variant) | 9 | 5 | 7.0 | 6 | 5 | 8.7 | 10 | 5 | 7.5 | 5 | 3 | 3.7 |
| q One thoracic vertebral center bipartite (variant) | 8 | 5 | 5.8 | 2 | 2 | 2.0 | 5 | 4 | 3.5 | 4 | 4 | 2.8 |
| r More than one thoracic vertebral center bipartite (variant) | 1 | 1 | 0.6 | 0 | 0 | 0.0 | 2 | 2 | 1.7 | 0 | 0 | 0.0 |
| s One lumbar vertebral center semi-bipartite (variant) | 1 | 1 | 0.6 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |

%† = Group mean percent per litter

NF = Number of fetuses

NL = Number of litters

%† = Group mean percent per litter

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Table 2 (continued)

Group Incidence of Fetal Skeletal Findings

| | Dose Level (mg/kg/day) | | | | | | | | | | | |
|--|----------------------------|----|------|-----|----|------|-----|----|------|------|----|------|
| | 0 (Control) | | | 100 | | | 300 | | | 1000 | | |
| | Number of Fetuses Examined | | | | | | | | | | | |
| | 126 | | | 121 | | | 133 | | | 139 | | |
| | NF | NL | %† | NF | NL | %† | NF | NL | %† | NF | NL | %† |
| Sternebrae 1-4 | | | | | | | | | | | | |
| t Incomplete ossification of one sternebra (variant) | 0 | 0 | 0.0 | 2 | 1 | 1.6 | 1 | 1 | 0.8 | 5 | 4 | 3.1 |
| u Two or more sternebrae fused | 1 | 1 | 1.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| v No ossification of one sternebra | 4 | 2 | 2.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| w No ossification of more than one sternebra | 1 | 1 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| x Incomplete ossification of more than one sternebra (variant) | 2 | 2 | 1.5 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0.5 |
| Total number of affected fetuses | 76 | 20 | 60.3 | 59 | 19 | 49.7 | 73 | 21 | 55.0 | 70 | 20 | 49.2 |

* = significantly different from control group <0.05

NF = Number of fetuses

NL = Number of litters

%† = Group mean percent per litter

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Table 3 Group Incidence of Fetal Skeletal Development

| | | Dose Level (mg/kg/day) | | | | | | | | | | | |
|--|--------|----------------------------|----|------|-----|----|---------------------|-----|----|--------|------|----|--------|
| | | 0 (Control) | | | 100 | | | 300 | | | 1000 | | |
| | | Number of Fetuses Examined | | | | | | | | | | | |
| | | 126 | | | 121 | | | 133 | | | 139 | | |
| | | NF | NL | %† | NF | NL | %† | NF | NL | %† | NF | NL | %† |
| Number of Ribs (left/right) | 13/10 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 0.7 |
| | 12/12 | 1 | 1 | 1.3 | 1 | 1 | 0.7 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| | 13/13 | 123 | 20 | 97.9 | 120 | 21 | 99.3 | 133 | 22 | 100.0 | 136 | 23 | 98.7 |
| | 14/13 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 0.6 |
| | 14/14 | 1 | 1 | 0.8 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| Number of fully Ossified Sternebrae | <4 | 6 | 3 | 4.2 | 0 | 0 | 0.0 | 0 | 0 | 0.0 | 1 | 1 | 0.6 |
| | 4 | 12 | 5 | 8.7 | 14 | 6 | 10.2 | 20 | 9 | 14.8 | 17 | 8 | 11.0 |
| | >4 | 108 | 19 | 87.1 | 107 | 21 | 89.8 | 113 | 22 | 85.2 | 121 | 23 | 88.3 |
| Number of Post Lumbar Vertebral Centra | <7 | 12 | 4 | 8.1 | 0 | 0 | *0.0 | 1 | 1 | *0.9 | 3 | 2 | *1.7 |
| | ≥7 | 114 | 19 | 91.9 | 121 | 21 | *100.0 | 132 | 22 | *99.1 | 136 | 23 | *98.3 |
| Number of Post Lumbar Vertebral Arches | <5 | 18 | 7 | 13.6 | 2 | 2 | 1.5 | 3 | 3 | 2.3 | 9 | 6 | 5.6 |
| | ≥5 | 108 | 19 | 86.4 | 119 | 21 | 98.5 | 130 | 22 | 97.7 | 130 | 23 | 94.4 |
| Number of Metacarpals | <6 | 2 | 1 | 1.3 | 2 | 1 | 2.4 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| | 6 | 34 | 13 | 25.3 | 33 | 16 | 30.3 | 44 | 17 | 30.7 | 36 | 17 | 27.4 |
| | >6 | 90 | 19 | 73.4 | 86 | 19 | 67.3 | 89 | 22 | 69.3 | 103 | 22 | 72.6 |
| Number of Forelimb Phalanges | <2 | 111 | 20 | 88.0 | 107 | 21 | **89.6 | 124 | 22 | *93.3 | 128 | 23 | **92.2 |
| | ≥2 | 15 | 8 | 12.0 | 14 | 7 | **10.4 | | 5 | *6.7 | 11 | 8 | **7.8 |
| Number of Metatarsals | 6 | 9 | 3 | 5.9 | 4 | 2 | 8.3 | 0 | 0 | *0.0 | 0 | 0 | *0.0 |
| | >6 | 117 | 20 | 94.1 | 117 | 20 | 91.7 ⁹ | 133 | 22 | *100.0 | 139 | 23 | *100.0 |
| Fontanelle Size | Small | 41 | 14 | 34.8 | 9 | 7 | **11.3 | | 4 | **5.9 | 20 | 7 | *15.8 |
| | Medium | 67 | 18 | 52.1 | 106 | 20 | **83.6 ⁸ | 114 | 22 | **85.8 | 112 | 21 | **79.2 |
| | Large | 18 | 8 | 13.1 | 6 | 5 | 5.1 ⁸ | 11 | 6 | 8.3 | 7 | 4 | 5.0 |

NF = Number of fetuses

NL = Number of litters

%† = Group mean percent per litter

* = significantly different from control group <0.05

** = significantly different from control group <0.01