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Polyethylene glycol-g-polyvinyl alcohol grafted copolymer: Reproductive toxicity study in Wistar rats

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ABSTRACT

Polyethylene glycol-g-polyvinyl alcohol (PEG–PVA) grafted copolymer was administered by gavage to groups of 25 male and 25 female young Wistar rats at doses of 0 (vehicle control), 100, 300, or 1000 mg/kg bw/day for one generation (F_0). The study followed the treated F_0 generation through mating, gestation, lactation, and weaning of the F_1 generation. F_1 animals were mated and followed to gestation day (GD) 15–17 at which time F_2 implants were evaluated. There were no indications from the various clinical and gross pathological examinations that the oral administration of PEG–PVA grafted copolymer to the F_0-parental rats produced any signs of general, reproductive, or developmental toxicity in the F_0 or F_1 animals or F_2 implants. Based on the lack of any dose-related or biologically relevant effects on fertility, reproduction, development, and overall health of rats gavaged with PEG–PVA grafted copolymer and their progeny, the no-observed-adverse effect level (NOAEL) was determined to be the highest dose tested of 1000 mg/kg bw/day.

1. Introduction

Polyethylene glycol-g-polyvinyl alcohol (PEG–PVA) copolymer (Kollicoat® IR) is a synthetic polymer comprised of approximately 25% polyethylene glycol (PEG) and approximately 75% polyvinyl alcohol (PVA) units. The spray-dried product contains approximately 0.3% colloidal silicon dioxide and is described in more detail in the Introduction to this special supplement. The excipient is mainly used in the production of instant-release tablet coatings for pharmaceutical and dietary supplement products.

As part of the safety evaluation program of Kollicoat® IR conducted by Experimental Toxicology and Ecology, BASF Aktiengesellschaft (Ludwigshafen/Rhein, FRG), reproductive toxicity was studied in rats over one generation.

2. Materials and methods

This study was conducted in accordance with the EC Commission Directive 91/507/EEC (Official Journal of European Communities L 270/32, September 26, 1991), the International Conference on Harmonization: Guideline on Detection of Toxicity to Reproduction for Medicinal Product, US Food and Drug Administration (ICH, 1994, 1995), and in accordance with the OECD principles of Good Laboratory Practice (GLP) (OECD, 1998). Permission for animal studies was obtained from the local regulatory agencies, and all study protocols were in compliance with the federal guidelines.

2.1. Test material

PEG–PVA grafted copolymer (CAS No. 96734-39-3; purity > 99%) used in these studies was provided by BASF Aktiengesellschaft (Ludwigshafen/Rhein, Germany) and was administered via gavage. Test substance solutions were prepared fresh weekly by adding the appropriate amount of test substance into a specified volume of distilled water and thoroughly mixed. Water concentrations were confirmed analytically at the beginning and toward the end of the treatment period. Gel permeation chromatography confirmed the stability of the test substance in water at room temperature after 7 days.

2.2. Animals

A total of 100 male and 100 nulliparous and non-pregnant female CrlGIxBrl-Han:WI Wistar rats (supplied by Charles River Laboratories, Germany) were randomly allocated by weight using the method of Nijenhuis and Wilf (1978) into groups of 25/sex and acclimated for 5–6 days. At the beginning of treatment, males were 76 ± 2 days old with a mean body weight of 287.1 (range: 268.9–304.3 g) and females were 82 ± 2 days old with a mean body weight of 189.7 (range: 176.9–202.7 g). Rats were individually housed in type DK III stainless steel wire mesh cages (supplied by Becker & Co., Castrop-Rauxel, Germany) with the exception of when male and female mating partners were housed together for overnight matings and when dams were housed with their litters (GD 18 to lactation day 21) in Makrolon type M III cages (supplied by Becker & Co., Castrop-Rauxel, Germany). Pregnant females were provided with cellulose wadding for nesting material toward the end of gestation. The bedding used throughout the study was SSNIFF type 3/4 (supplied by SSNIFF Spezialdiäten GmbH, Soest, Germany). Room temperature

Abbreviations: GD, gestation day; PEG–PVA, polyethylene glycol-g-polyvinyl alcohol; PEG, polyethylene glycol; PVA, polyvinyl alcohol.

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was maintained at 20–24 °C with a relative humidity of 30–70% and a 12-h light/12-h dark cycle. Feed and drinking water were provided *ad libitum* throughout the study.

3. Study design

Groups of Wistar rats (25/sex/group; F_0 generation) were gavaged daily with 0 (vehicle control), 100, 300, or 1000 mg PEG–PVA grafted copolymer/kg bw (dose volume = 10 ml/kg bw). After 28 days of treatment for male animals and 14 days for female animals, males and females from the same dose group were mated overnight on a 1:1 pairing for a maximum period of 2 weeks. Each male was mated with a predetermined female from the same dose group throughout the mating period. It was necessary to deviate from the 1:1 mating of the F_0-generation animals three times during the study: 1 male from the control, 1 male from the low-dose, and 2 males from the mid-dose groups were found dead after gavage error and other males from the corresponding control or test group were mated with the respective females (i.e., a few males were mated with 2 females instead of just 1 female). After mating, if vaginal smears were positive for sperm, the female was presumed pregnant. This day was designated "GD 0". Males were euthanized after the mating period. Females continued to receive treatment and were allowed to birth and rear their pups (F_1 generation) until day 21 after parturition. After weaning of F_1-generation pups, F_0-generation females were euthanized. Groups of F_1-generation pups (25/sex/group) were selected for F_1-generation parental animals by lot during rearing, attempting to take each litter into account. All other F_1-generation pups were euthanized after weaning. F_1-generation parental animals did not receive any treatment post-weaning. At least 103 days after assignment of the F_1-parental animals, males and females were mated as described for the F_0-parental animals. During the mating period, one mid-dose male was found dead and was replaced by another mid-dose male. After the mating period, male F_1-parental animals were euthanized. F_1-Parental females were euthanized on GD 15–17 and the content of their uteri (i.e., F_2-implants) was evaluated. An overview of the study design is shown in Fig. 1. The F_1-parental animals were never directly exposed to the test substance; however, exposure to the test substance or its metabolites might have occurred in utero and/or via the milk of their F_0 mothers.

3.1. Clinical examination and examination of reproductive performance

3.1.1. Parental animals

All parental animals were checked at least once a day for mortality and clinically overt signs of toxicity. Feed consumption was determined weekly during pre-mating, gestation, and lactation but not between days 14 and 21 after parturition, since during this time pups were beginning to consume considerable amounts of feed. Body weights of both sexes were recorded on the first day of the premating period and then twice a week until the end of the study. Gestating and lactating animals were weighed daily.

Estrous cycles were evaluated daily for all F_0- and F_1-parental rats for a minimum of 1 week prior to mating and during the mating period until the female exhibited evidence of mating. Mating partners, the number of mating days until vaginal sperm could be detected in the female, and the gestational status of the female were noted for F_0 and F_1 breeding pairs. For F_0-parental females, the mating index (division of the number of females mated by the number of females placed with males), fertility index (division of the number of females pregnant by the number of females mated), gestation index (division of the number of females with live pups by the number of females pregnant), and live birth index (division of the number of liveborn pups at birth by the total number of pups born) were calculated. Implantations of the F_0 dams were counted and post-implantation loss calculated by dividing the difference between the number of implantations and the number of pups delivered by the number of implantations. For the F_1-parental females, the number of corpora lutea, and the number and distribution of implantation sites were determined. Implantation sites were classified as live fetuses and dead implantations (early resorptions, late resorptions, and dead fetuses). Conception rate and pre- and post-implantation losses were calculated. The conception rate was obtained by dividing the number of pregnant females by the number of fertilized animals, pre-implantation loss was the difference between the number of corpora lutea and the number of implantations divided by the number of corpora lutea, and the post-implantation loss was calculated by dividing the difference between the number of implantations and the number of live fetuses by the number of implantations. For mated males, the mating index (division of the number of males with confirmed mating by the number of males placed with females) and fertility index (division of the number of males impregnating females by the number of males placed with females) were calculated. All values were expressed in percentage by multiplying the ratio by a factor of 100.

At necropsy, the right testis and cauda epididymis were removed from the F_0 and F_1 males of all groups. Sperm motility, morphology, and head count from cauda epididymus and testis were evaluated using the microscopic methods of Slott et al. (1991) and Feuston et al. (1989). Motility and head count from testis were determined in all groups; whereas morphology and head count from cauda epididymus were determined from control and high-dose groups only.

3.1.2. Pups/litters

All pups derived from the F_0 parents were examined on the day of birth to determine the total number of pups and the number of liveborn and stillborn members of each litter. Litters were observed daily for any overt signs of toxicity and any dead or

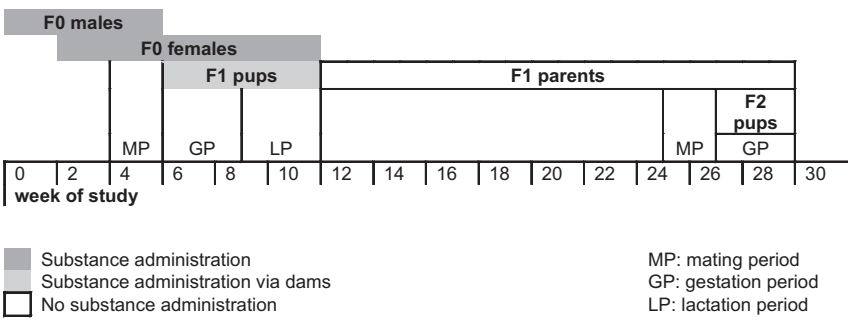

Fig. 1. Reproductive toxicity study design in Wistar rats.