REACH

Octocrilene

EC number: 228-250-8 | CAS number: 6197-30-4

Toxicological information

Endpoint summary

Administrative data

Key value for chemical safety assessment

Effects on fertility

Description of key information

An Extended One Generation Reproductive Toxicity Study (EOGRTS) according to OECD 443 and GLP is chosen as the key study:

- NOAEL for parental systemic toxicity = 2100 ppm or based on effects on body weights.
- NOAEL for fertility/reprod. performance = 2100 ppm (overall mean: 153/163 mg/kg bw/d for males/females) based on lower number of implantation sites and lower number of pups delivered. The differences in reproductive parameters occurred at a maternally toxic dose level.
- NOAEL for general and sexual development = 2100 ppm (overall mean: 153/163 mg/kg bw/d for males/females) based on the effects on pup body weights.
- NOAEL for neuro-/development = 7000 ppm (overall mean: 534/550 mg/kg bw/d for males/females) based on the absence of effects

Additional information

An Extended One Generation Reproductive Toxicity Study (EOGRTS) in rats with octocrilene was performed according to OECD 443 and GLP and is chosen as the key study for the endpoint reproductive toxicity.

In the preceding dose-range finding study, Wistar rats were treated with octocrilene via dietary exposure during a premating period of 4 weeks, during mating (one week) and post-mating up to the day of sacrifice for male rats and during a premating period of 4 weeks, during mating (one week), gestation and lactation until (or shortly after) postnatal day 21 for female rats (BASF 85R0495/00X057). The concentration of octocrilene in the diets was 0, 5000 and 15000 mg/kg diet for the control, low- and high-dose groups, respectively. These concentrations were halved during the lactation phase due to the considerable higher food consumption of the dams during this phase. The test substance intake during the entire study ranged from 279-399 and 812-1271 mg/kg bw/day in males as well as 323-618 and 796-1740 mg/kg bw/day of females in the low- and high-dose group, respectively.

There was no treatment-related mortality. In male animals and in female animals during the premating period, daily clinical observations did not reveal any treatment-related clinical signs. During the gestation and lactation period, the incidences of female animals of the high-dose group showing piloerection was 5 /10 and 6 /10, respectively. Body weights and body weight gains were markedly decreased in the high-dose group in both sexes (reductions by 11%/33% (males) and 21%/38% (females, gestation) in body weight/-gains, respectively). Slight, but statistically significant, effects on these parameters were also seen at the low dose group.

Food consumption was decreased in the high-dose group in both sexes. Food consumption in the animals of the low-dose group was comparable to controls.

Hematological and clinical chemistry examinations revealed lower hemoglobin concentrations and eosinophil counts in females of the high dose group. Males of the high dose group showed higher albumin concentrations. Increased GGT activities as well as higher urea values were observed in females of the high dose group. Higher urea values were also measured in females of the low-dose group.

Liver weights were dose-dependently increased in males and females (approx. +20% and +45% relative weights in low and high dose groups compared to controls, respectively). Higher thyroid weights were observed in both sexes of both treatment groups (approx. +30%/5% and +45%/25% relative weights of males/females in low and high dose groups compared to controls, respectively). Microscopically, an activated appearance of the thyroid was apparent.

Lower mean numbers of implantation sites and pups per litter were observed in the high-dose group (implantation sites: 9.7 vs. 12.7 in controls; pups: 8.9 vs. 12.1 in controls). Birth weight and postnatal body weight development of male and female pups of the high-dose group was decreased (approx. -30% on PND21). No compound-related effects on these parameters were observed in the low-dose group.

The results of this dose-range finding study were used for dose selection for the EOGRTS with the same test substance. A dose of 15000 mg/kg diet was considered too high as a high dose in the subsequent EOGRTS. Based on the results of this range-finding study, the selected concentrations of the test substance for the Extended One-Generation Reproductive Toxicity Study with octocrilene in rats were set to 0, 750, 2100 and 7000 mg/kg diet.

In the main EOGRTS, octocrilene was applied via oral administration of the determined dietary concentrations (750 ppm, 2100 ppm and 7000 ppm) to male and female rats during a premating period of 10 weeks and during mating (max. 2 weeks), gestation and lactation until postnatal day 21 (BASF 2019; 03R0495/00X056). At weaning, pups were distributed to different cohorts and were exposed to the same dose levels of the test substance as their parents during their growth into adulthood. Cohorts 1A and 1B of this study assessed reproductive performance and Cohorts 2A and 2B focused on neurodevelopmental endpoints. Animals of Cohort 1B were used for breeding a second generation. The overall mean dose of octocrilene was calculated to be 55/58, 153/163 and 534/550 mg/kg bw/d in the low, mid and high dose group of males/females, respectively.

General observations

In total, two animals (one high-dose male animal of Cohort 1A and one high-female of Cohort 1B) were sacrificed in a moribund condition. The macro- and micro-observations in these animals were not considered to be related to treatment. Furthermore, no treatment-related (detailed) clinical signs were observed during the study.

Body weights of the male and female animals of the high-dose group were lower than the corresponding control animals throughout almost the entire study. The first and the second parental generation showed decreased body weights before and during pregnancies (down to -7% in F0 males and down to -11% (premating), -13% (gestation) and -11% (lactation) in F0 females; down to -8% and -14% in F1 -cohort 1A males and females, respectively). In general, food consumption of the male and female animals of the high-dose group was slightly but statistically significantly lower than of the control animals (during the F0 -generation maximally -10% in males and -19% in females, and in F1-Cohort 1A maximally -6% in males and -15% in females). The observed effects on body weight and food consumption in the high-dose group were considered treatment related.

Except for the increase in gamma glutamyl transferase activity (GGT) in male and female animals of the high-dose group of both the F0- and Cohort 1A F1-generation, no treatment-related effects were observed on hematology, clinical chemistry and urinalysis in F0- generation and Cohort 1A animals.

The decreased terminal body weights (~5 to 10% below controls) and the increased relative liver (males ~20%, females ~30%)) and thyroid weight (males ~25 to 30%, females F0 ~25%) as observed in male and female animals of the high-dose group of the F0- and Cohort 1A F1-generation were considered treatment related. The decreased terminal body weights were considered as adverse, whereas the increased liver and thyroid weights were considered to be adaptive changes in rats. No effects were observed on the weight of the reproductive organs of Cohort 1B F1-generation animals.

At necropsy of F0-generation and Cohort 1A F1-generation animals, no treatment-related macroscopic changes were observed. In F0-generation animals of the mid- and high-dose group and in Cohort 1A animals of the high-dose group an increased incidence of activated appearance of the thyroid gland in comparison with the controls was observed. It was characterized by loss of colloid from the follicles and hypertrophy and hyperplasia of follicular epithelial cells. These findings were considered treatment related but adaptive changes in rats.

Fertility and reproductive parameters

No treatment-related effects were observed on estrus cycle related parameters in female animals of the F0-generation and in animals of Cohort 1A of the F1-generation. No treatment-related effects were observed on epididymal and testicular sperm parameters in male animals of the F0-generation and in animals of Cohort 1A of the F1-generation. No treatment-related effects were observed on fertility and reproductive performance of male and female animals of the F0-generation and of Cohort 1B of the F1-generation. No effects were observed on TSH and T4 analysis in animals of the F0-generation and in adult F1-generation animals of Cohort 1A. No effects were observed on T4 concentrations in serum of F1- and F2-generation pups culled on PN day 4 and, in addition, no treatment-related effects were observed on T4- and TSH concentrations in serum of F1- and F2-generation pups sacrificed on PN day 21.

Lower mean number of implantation sites, and consequently, a lower number of pups delivered in female animals of the high-dose group of the F0-generation (implantation sites: 10.7 versus 12.3 in controls; pups: 9.6 versus 11.4 in controls) and of Cohort 1B of the F1- generation (implantation sites: 9.3 versus 10.7 in controls; pups: 9.3 versus 10.3 in controls) were observed. The decrease in the mean number of implantation sites was slight, not statistically significant and the mean numbers of the F0-generation were within the historical control range (HC range: 9.8-12.7). However, due to the fact, that this decrease was observed in both generations and in the range finding study (see BASF 85R0495/00X057), the findings were considered treatment related and adverse.

Maternal stress exposure and related effects on the developing embryo during the preimplantation period has been described in literature. In a study with pregnant ICR mice, maternal restraint stress (3 times a day for 30 minutes from day 1 to day 4 of pregnancy) resulted in significantly increased serum corticosterone concentrations (Burkus et al., 2015). Further, a significant reduction in implantation sites *in uteri* was observed on gestational day 6 when compared with untreated controls. The blastocysts of stressed mothers showed reduced average cell numbers in the trophoectoderm and the inner cell mass lineages, which was indicated to contribute to the factors responsible for the decreased implantation rate.

Maternal preimplantation stress and its effects on implantation sites and pup numbers were investigated by comparing inhouse historical control data on Wistar rats, i.e. the strain used in the EOGRTS. The database consists of 80 studies (1787 litter) of time mated and subsequently transported rats and 35 studies (949 litter) with inhouse mated rats without further transportation (study dates 2013-2018). The time mated and transported rats (mean = 10.8; Range 9.4 – 11.9) showed a reduction of 1.4 in implantation sites when compared to inhouse mated animals (mean = 12.2; Range 9.4 – 13.9). Consequently, the difference in the mean number of delivered pups was 1.5 (transported: mean = 10.1; Range 8.5 – 11.2 versus inhouse mated: mean = 11.6 (Range 9.9 – 12.7). These data further substantiate a dependency of both parameters to maternal stress conditions due to transportation of pregnant animals, which is of a comparable magnitude to the changes observed after administration of octocrilene at a dose of 7 000 ppm. Since the octocrilene dependent changes in these reproductive parameters occurred at a maternally toxic dose level, a dependency between these effects cannot be excluded. However, more mechanistic information is required to elucidate, if the events leading to the decrease in implantation sites occur before fertilization (referring to an impairment of female fertility such as ovulation or ovular transport) or during early development including impairment of implantation. Therefore, the registrant proposes an additional reproductive toxicity study in Wistar rats with a comparable octocrilene application 10 weeks before mating until completion of the implantation phase (i.e. gestational day 7). Assessment of the mating and fertility parameters, the corpora lutea and implantation sites would allow to better characterize the type of effect observed.

General and sexual developmental parameters

The mean number of live pups on postnatal day 0 was lower in the high-dose group of the F0- generation and in the high-dose group of Cohort 1B of the F1-generation. This finding was considered as related to the lower number of implantation sites and the lower number of pups delivered as observed in the animals of these groups. The effects were considered treatment related. No other relevant effects were observed on implantation loss, stillborn pups, dead, missing and/or cannibalized pups, litter loss, pup viability indices and sex ratio. No treatment-related effects were observed on clinical signs of pups nor on macroscopic observations at sacrifice and of dead pups in F1-generation pups and Cohort 1B F2-generation pups.

Overall, in the high-dose group, the body weight of F1-generation pups and of Cohort 1B F2-generation pups was lower than of the corresponding control pups (~10% on postnatal day 21), which is considered due to relatively high compound intake via initiating food uptake. This finding was considered to be related to treatment. Preputial separation (control: 43.0 days, high dose 46.4 days), vaginal opening (control: 31.4 days, high dose: 33.9 days), and first estrus stage occurred later in Cohort 1A-generation offspring. However, these differences were not considered as delayed sexual development but as consequence of delayed general development (lower pup weights). No direct effects were observed on organ weights of F1-generation pups.

No effects were observed on nipple retention in male F1-generation pups and Cohort 1B F2-generation pups. No treatment-related effects were observed on the development of the ovarian follicles from primordial small follicles into corpora lutea. No treatment-related effects were observed on splenic lymphocyte subpopulation analysis in Cohort 1A F1-generation animals.

Neuro(developmental) parameters

Functional observation battery (FOB) and spontaneous motor activity analysis did not reveal any effect of octocrilene in animals of Cohort 2A of the F1-generation. The results of the auditory startle response did not indicate any neurotoxic potential of octocrilene in animals of Cohort 2A of the F1-generation. No treatment-related adverse effects were observed on brain weight, brain length and brain width in animals of Cohort 2A and Cohort 2B, respectively. Brain morphometric analysis of the thicknesses of 10 major regions of the brain did not reveal any compound-related adverse effect. Macroscopic observations at sacrifice of animals of Cohort 2A and Cohort 2B did not reveal any treatment-related abnormalities. Furthermore, microscopic observations of brains and neuronal tissues of animals of Cohort 2A and brain of animals of Cohort 2B showed no treatment-related abnormalities.

Conclusion:

Based on the effects on body weights the NOAEL for parental effects was placed at the mid-dose concentration of 2100 mg test substance per kg diet.

Based on the lower number of implantation sites and the lower number of pups delivered, the NOAEL for fertility and reproductive performance was placed at the mid-dose concentration of 2100 mg test substance per kg diet (overall mean: 153/163 mg/kg bw/d for males/females). The differences in reproductive parameters occurred at a maternally toxic dose level.

Based on the effects on pup body weights the NOAEL for general and sexual development was placed at the mid-dose concentration of 2100 mg test substance per kg diet (overall mean: 153/163 mg/kg bw/d for males/females).

There were no effects of the test item on neuro(developmental) parameters. The NOAEL for neuro(developmental) parameters was placed at the high-dose concentration of 7000 mg test substance per kg diet (overall mean: 534/550 mg/kg bw/d for males/females).

Further supportive studies addressing specific aspects of reproductive toxicity/fertility are available for octocrilene.

In a supportive uterotrophic assay in immature female Wistar rats (age 20 ± 1 days), octocrilene in corn oil was administered orally to 10 animals per dose group (250 and 1000 mg/kg bw/d) for 3 consecutive days (BASF 2001; 07R0228/99121). Test substance administration led to statistically significantly retarded body weight gain at 1000 mg/kg bw/d, being regarded as sign of systemic toxicity, however no increase the uterine weights were observed at any dose level. Histopathologically, no changes were detected in the uterus of the octocrilene treated animals. Taken together, octocrilene showed no uterotrophic (estrogenic) effect in rats under the chosen testing conditions, when compared with the carrier control.

In a supportive study according to the OECD Protocol and Guidance for the Conduct of the Rodent Hershberger Assay (Phase 2 of the Validation of the Rodent Hershberger Assay) and GLP, octocrilene was administered in corn oil via gavage to groups of 6 castrated but Testosterone propionate (0.4 mg/kg) substituted male Wistar rats for 10 days at dose levels of 300 and 1000 mg/kg bw/day (BASF 2003; 47S0495/00149).

Substance related findings in the high dose group (1000 mg/kg bw/day) were increases of absolute and relative liver weights, decreases of absolute and relative ventral prostate weights and weights of the muscle bulbocavernosus / levator ani. In the low dose group (300 mg/kg bw/day), absolute and relative liver weights were significantly increased. No octocrilene related effects in clinical examinations, on hormone levels

(testosterone, dihydrotestosterone and luteinizing hormone) and the histology of the prostate, seminal vesicle and the bulbo-urethral gland were observed when compared to animals given testosterone propionate only.

The observed decrease in organ weights of ventral prostate and the muscle bulbocavernosus together with the levator ani may have been fortuitous or is to be explained by an enzyme induction, indicated by the observed increased liver weights connected with a higher metabolism rate of the substituted androgen testosterone propionate. In contrast, absolute and relative weights of the other accessory sex organs were not significantly reduced. Moreover, the histology of prostate, seminal vesicle and the bulbo-urethral gland was comparable to the control. Therefore, under the conditions of the present study, regarding clinical examinations, hormone investigations as well as pathological evaluations, no indication for an antiandrogen efficacy of octocrilene was determined.

In a supportive oral subchronic repeated dose study according to OECD TG 408 and GLP, octocrilene was administered for 90 days at doses of 750, 2250, 4500 and 15000 ppm to 10 Wistar rats per sex and dose in the diet, corresponding to approx. 58, 175, 340, 1085 mg/kg bw/day ingested test substance, respectively (BASF 1993; 50S0227/92059). No changes in absolute and relative testes weights in males and adrenal weights in males/females were observed. Furthermore, no treatment related microscopic findings were observed in adrenal glands, epididymides, prostate and testes of male animals and in adrenal glands, mammary gland, ovaries, uterus and vagina of female animals.

A treatment related slight or moderate hypertrophy of the follicular epithelium of the thyroid gland, associated with minimal or slight pale staining colloid was observed in the two high dose groups. Based on the indications observed for hepatic enzyme induction (see Chapter 7.5.), these enzymes are assumed to be responsible for metabolization of thyroid hormones, resulting in an increased removal of circulating T3 and T4 with subsequent elevation of thyroid stimulating hormone secretion (see also BASF 99C0495/00S048). The incidence of hypertrophic cells in the pituitary gland tended to occur in the middle of the pars distalis rather than in the lateral regions suggesting that they may be so called thyroidectomy cells caused by interference in the homeostatic feedback mechanism. Taken together, the observed effects on thyroid and male pituitary gland are considered to have occurred as a secondary consequence of hepatic enzyme induction.

In a dermal subchronic study, reported as summary in a publication, 5 male New Zealand white rabbits per group were treated topically (open) with octocrilene (130, 264, 534 mg/kg/day) in a mixture of petrolatum and C12-15 alkylbenzoate for 13 weeks (5 days per week, 65 applications in total; Odio 1994). Specific investigation of male reproductive parameters revealed no signs of test substance associated histopathological abnormalities in the testes and epididymides. Furthermore, epididymal sperm concentration and sperm motility was not significantly affected compared to control animals.

References:

BURKUŠ J. et al., 2015. Stress exposure during the preimplantation period affects blastocyst lineages and offspring development. Journal of Reproduction and Development, Vol. 61, No 4.

Effects on developmental toxicity

Description of key information

In a prenatal toxicity study in rats according to OECD TG 414 and GLP, octorilene caused some slight

effects in the dams at 1000 mg/kg bw/day, and a marginal increase of the relative liver weight at 400 mg/kg bw/day. A dosis of 100 mg/kg bw/day was tolerated by the dams without any substance-induced findings. No signs of embryo-/ fetotoxicity were noted up to and including the dose of 1000 mg/kg bw/day.
Based on the chosen key study, the no observed adverse effect level for dams is set at 100 mg/kg bw/day and the no observed adverse effect level for the fetus is set at 1000 mg/kg bw /day.
Further supportive evidence from a developmental toxicity study after dermal application of octocrilene in rabbits and after oral application in mice confirmed the absence of test substance related developmental toxicity in other species than the rat.

Additional information

In the chosen key study for developmental toxicity, octocrilene has been tested in Wistar rats (23 - 25 pregnant female rats per group) for prenatal toxicity according to OECD TG 414 and GLP (BASF 30R0227/92063). The test substance has been administered in olive oil DAB 10 via gavage at doses of 100, 400 and 1000 mg/kg bw/day on day 6 through day 15 post coitum.

As test substance related findings in the dose group receiving 1000 mg/kg bw/day, transient salivation shortly after test substance administration was observed in most of the animals together with transient reddish-brown discoloration of the fur in the anogenital region or urine smeared fur in a few animals during several days of the treatment period. Furthermore, slight but statistically significant increase in absolute and relative liver weight (approx. 9% above controls) was observed.

In the dose group receiving 400 mg/kg bw/day, a marginal but statistically significant increase in relative liver weights (approx. 6% above controls) was observed.

In the low dose group (100 mg/kg bw/day) no test substance-related effects on dams were observed.

There were no indications of any substance-induced embryo-/fetotoxicity and especially no signs of any teratogenicity in the present full-scale prenatal toxicity study up to and including the dose of 1000 mg/kg bw/day.

Thus, under the conditions of this full-scale study, octorilene caused some slight effects in the dams at 1000 mg/kg bw/day, and a marginal increase of the relative liver weight at 400 mg/kg bw/day. A dosis of 100 mg/kg bw/day was tolerated by the dams without any substance-induced findings. No signs of embryo-/ fetotoxicity were noted up to and including the dose of 1000 mg/kg bw/day.

Based on the chosen key study, the no observed adverse effect level for dams is set at 100 mg/kg bw/day and the no observed adverse effect level for the fetus is set at 1000 mg/kg bw /day.

Further evidence from other species, i.e. a developmental toxicity study after dermal application of octocrilene in rabbits and after oral application in mice confirmed the absence of test substance related developmental toxicity in other species than the rat.

A percutaneous developmental toxicity study is available as summary in a publication using New Zealand White does, treated topically with octocrilene in a mixture of petrolatum and C1-15 alkylbenzoate (65, 267 mg/ kg/day) on days 6 through 18 of gestation (Odio 1994). Body weights, food consumption and further maternal parameters including clinical observations and gross necropsy of thoracic and abdominal viscera, uterus and ovaries were comparable between treated and control animals. Female reproductive parameters addressed by examinations of ovaries and uterine content were unaffected by treatment with octocrilene. One doe per octocrilene dose group aborted, being statistically indifferent by respective historical control data. Offspring parameters, covering mortality, survival rates, gender ratios, litter sizes and weights were comparable between treated and control animals, and external, soft tissue, skeletal and head examination yielded no evidence of octocrilene-associated teratological effects.

In the same publication, an in vivo teratology screening study in CD-1 mice according to Chernoff-Kavlock was reported, where octocrilene (100, 300, 1000 mg/kg bw/d) was administered in corn oil via gavage on days 8-12 of gestation (Odio1994). All dams survived offspring delivery and maternal body weight changes were comparable across all treatment groups. Pregnancy rates were lower in the high dose group, which is not attributable to the test substance, being applied after mating. Litter size or numbers of live and dead pups delivered were indifferent between the test groups. Postnatal survival and body weight gain of pups were unaffected and an observed trend toward decreased survival of pups born to octocrilene-treated compared to control dams did not achieve statistical significance.

For effects of octocrilene on general and sexual and neuro-developmental parameters in the EOGRTS (BASF 2019; 03R0495/00X056), see additional information on "Effects on fertility".

Justification for classification or non-classification

Based on the available data, the endpoint is currently assessed as inconclusive.

Additional information