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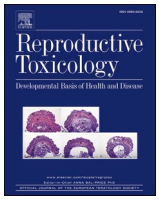

Prenatal developmental toxicity evaluation of higher olefins in Sprague-Dawley rats

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ABSTRACT

Higher olefins (HO) are used primarily as intermediates in the production of other chemicals, such as polymers, fatty acids, plasticizer alcohols, surfactants, lubricants, amine oxides and detergent alcohols. The potential prenatal developmental toxicity of five HO (i.e. Hex-1-ene, Nonene, branched, Octadec-1-ene, and Hydrocarbons, C12–30, olefin-rich, ethylene polymn. by product) were evaluated in prenatal development toxicity studies (OECD TG 414 (2001)) in Sprague-Dawley rats as part of the regulatory requirements for REACH registration. In each study, the HO were administered by gavage at dose levels of 0, 100, 300 and 1000 mg/kg bw/day from Day 3 to Day 19 of gestation. Maternal food consumption, body weights, and clinical signs were monitored throughout gestation. The rats were sacrificed on Day 20 of gestation and examined for standard parameters of reproductive performance (number of corpora lutea, number of implantations, pre- and post-implantation loss, number of live- and dead fetuses, sex-ratio and the weight of the reproductive organs). The fetuses were weighed and examined for external, visceral, and skeletal variations and malformations. The results from these studies showed that none of the HO treated groups showed maternal or embryo–fetal toxicity. Although occasionally incidental skeletal and visceral malformations were observed with Hex-1-ene and Octadec-1-ene, these findings were found to be spontaneous, unrelated to treatment and not indicative for any disturbance of fetal development. In conclusion, the No-Observed-Adverse-Effect Level (NOAEL) for all tested HO was determined to be 1000 mg/kg bw/day, which is the highest dose level administered, for both maternal and developmental toxicity.

1. Introduction

Higher olefins (HO) are highly valuable commercial chemicals and are used primarily as intermediates in the production of other chemicals (including polymers, fatty acids, mercaptans, plasticizer alcohols, surfactants, additives for lubricants, amine oxides and amines, non-ionic and alcohol detergents, and hydraulic fluids and additives) [1–3]. Most HO have production volumes of more than 1000 ton per year. To comply with the Registration, Evaluation, Authorisation and Restriction of CHemicals (REACH) (EC 1907/2006), which aims to improve the protection of human health and the environment through better and earlier identification of the intrinsic properties of chemical substances, information as described in Annexes VII – X needs to be provided [4,5].

Generally, HO are hydrocarbons with six to more than thirty carbons and a double-bond between two of the carbons (i.e. with a sum formula of C_nH_{2n}) [6]. Based on the position of the double-bond and the degree of branching, 4 types of HO can be distinguished: 1) linear alpha olefins (i.e. vinyl compounds - straight chain with a single double-bond in the alpha position); 2) linear internal olefins (i.e. cis/trans disubstituted - straight chain with a single double-bond in an internal position); 3) branched alpha olefins (i.e. vinylidene compounds - isomerized olefins with a single double-bond in the alpha position); and 4) branched internal olefins (i.e. trisubstituted or tetrasubstituted - isomerized olefins with a single double-bond in an internal position). Nine HO have been

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evaluated to provide information for the Screening Information Data Set (SIDS) under the High Production Volume chemicals program [7,8]. In general, HO appear to have a low order of acute toxicity by the oral, dermal and inhalation routes of exposure. For example, olefins with carbon number ranging from C_6 to C_{24} (alpha and internal, linear and branched) showed rat oral LD50 values > 5000 mg/kg, rat 4-hr inhalation LC50 values ranging = from 110 mg/L (32,000 ppm) to 6.4 mg/L (693 ppm) for C_6 to C_{16}, and rat/rabbit dermal LD50 values > highest doses tested (1430–10000 mg/kg). However, similar to the observations with other hydrocarbons of this carbon chain length, repeated dermal exposure may cause skin irritation by defatting which is a generic phenomenon with repeated exposure to hydrocarbons [9]. In addition, eye irritation and skin sensitization studies indicate that C_6- C_{18} HO are only slightly irritating to rabbit eyes and do not cause skin sensitization in guinea pigs [8]. Based on the available data, HO are not genotoxic based on the results of a battery of genetic toxicity assays, including the Ames bacterial mutagenicity test, the mouse lymphoma mammalian cell mutagenicity assay, mitotic gene conversion tests in yeast, chromosome aberration assays, mammalian cell transformation test and the unscheduled DNA synthesis assay [8].

Systemic toxicity of HO administered by the oral route has been well characterized in our previously published OECD 422 (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test) and OECD 408 (Repeated Dose 90-day Oral Toxicity) studies in Han Wistar rats [10,11]. In total, seven HO, which have carbon number ranging from C_8 to C_{24} and included all four types of HO, have been tested in either one or both of these studies. At the highest tested dose-level (500 or 1000 mg/kg bw/day), some effects were noted in animals of both sexes following treatment with HO that have a carbon number less than C_{18}, such as increased post-dosing salivation, and effects of the liver weights. In addition, increased kidney weight was also observed in males, but not females, from all dose groups. However, these effects were considered as adaptive changes of low toxicological concern and caused by the unpalatability of the test item formulation or the slight irritant qualities of the formulation rather than being attributable to systemic toxicity. Therefore, based on these findings, the no observed adverse effect level (NOAEL) for systemic toxicity of these higher olefins was determined to be at the highest tested dose in rats.

With regard to reproductive and developmental toxicity of HO, only screening studies are available (i.e. OECD 421/422). In combination with all publicly available data, including our previously published OECD 422 studies, there are a total of nine HO with carbon numbers ranging from C_6 to C_{18} (i.e. Hex-1-ene, Alkenes C6, Oct-1-ene, Nonene, branched, Decene, Tetradec-1-ene, Hexadecene, Octadec-1-ene, and Octadecene) that have been tested [10,12–15]. At a dose level of 1000 mg/kg bw/day, all HO (except Nonene, branched) showed no toxicologically meaningful differences in reproductive organ weights or histopathology in repeated dose toxicity studies, nor were any other reproductive and developmental parameters affected. Therefore, the NOAEL for reproductive and developmental toxicity was set at 1000 mg/kg bw/day for the HO mentioned above. However, there was one exception as females treated with 1000 mg/kg bw/day Nonene, branched showed a reduction in litter size and litter weights on Day 4 *post partum*. In addition, one female from this treatment group also showed a total litter loss between Days 2 and 4 *post partum*, and body weight gains in offspring which survived to Day 5 *post partum* were reduced between Days 1 and 4 *post partum* at this treatment level.

To date, no reports on the potential of HO to impair the development of the progeny have been published. General concern has been raised from our previous OECD 422 studies that Nonene, branched might pose a developmental risk [10]. Therefore, five prenatal development toxicity studies on Hex-1-ene, Nonene, branched, Octadec-1-ene, Octadecene and Hydrocarbons C12–30, olefin-rich, ethylene polymn. by product were conducted according to the OECD TG 414 [16]. The purpose of the study was to provide a better basis for the risk assessment of any adverse effects caused by HO on the dams and more importantly on the development of the embryo and fetus. In addition, the current studies provide strong source data for the read-across within the HO category, hence potentially reducing the numbers of animals that would be required to fulfil REACH information requirements if individual category members were to be separately tested.

2. Materials and methods

Studies in the current report were designed to be compatible with the Organization for Economic and Co-operation and Development (OECD) Guidelines for Testing of Chemicals, No 414 "Prenatal Developmental Toxicity Study" (adopted 22 January 2001). In addition, the study was designed and conducted to cause a minimum of suffering or distress to the animals consistent with the scientific objectives and in accordance with the Harlan Laboratories Limited, Shardlow, UK policy on animal welfare and the requirements of the United Kingdom's Animals (Scientific Procedure) Act 1986 Amendment Regulations 2012.

2.1. Chemicals and dosing

Five test materials Hex-1-ene (CAS 592–41–6), Nonene, branched (CAS No. 97280–95–0), Octadec-1-ene (CAS 112–88–9), Octadecene (CAS 27070–58–2), and Hydrocarbons, C12–30, olefin-rich, ethylene polymn. by product (CAS 68911–05–7) were obtained commercially. A full list of test materials details is provided in Table 1.

Dose levels were chosen based on the available data including a rat fourteen-day range-finding study during the OECD 422 studies [10]. In our previous studies, a high dosage of 1000 mg/kg bw/day was well tolerated and was considered acceptable as a high dosage for the investigation of pre-natal toxicity according to the OECD 414 guidelines (Fig. 1) [16]. Arachis Oil was used as the vehicle in our previous studies and it was also used for the current study. Hence, the dose levels used in current studies were 0, 100, 300 or 1000 mg/kg bw/day.

2.2. Animals

A total of ninety-six time-mated female Sprague-Dawley rats (166–284 g) were obtained from Charles River (UK) Limited and housed individually in polypropylene cages with stainless steel mesh lids and softwood flakes bedding. The animals had free access to a pelleted diet (Rodent 2018 C Teklad Global Certified Diet) and mains drinking water from polycarbonate bottles. Environmental enrichment included wooden chew blocks and cardboard tunnels. The diet, water, bedding, and enrichment materials were contaminant-free to ensure study integrity.

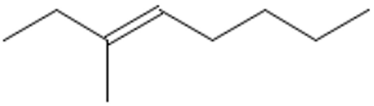
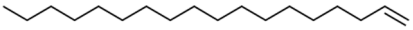
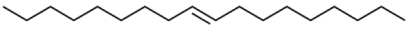
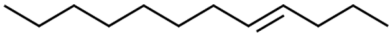
The rats were housed in an air-conditioned room at Harlan Laboratories Ltd., Shardlow, UK, with at least fifteen air changes per hour and controlled lighting (12 hours light/dark). Environmental conditions (22 ± 3 °C and 50 ± 20 % humidity) were monitored and maintained without deviation. Rats were randomly allocated to treatment groups based on stratified body weight and uniquely identified by ear punching.

2.3. Study design and parameters evaluated

The pregnant rats were randomly divided into four groups (N = 24), namely control, low, intermediate, and high which received either vehicle alone, 100, 300 or 1000 mg/kg/bw HO daily, from Days 3 (Days 5 for Octadecene and Hydrocarbons C12–30, olefin-rich, ethylene polymn. by product) to Days 19 of gestation, by gavage. The volume of test and control material administered to each animal was based on the most recent scheduled body weight and was adjusted at weekly intervals.

All animals were observed for overt signs of toxicity, ill-health and behavioral changes once daily during the gestation period. Additionally, during the dosing period, all observations were recorded immediately before, soon after dosing, and one hour post dosing.

Table 1
Test materials administrated in OECD 414 rats.

Test item	CAS No.	Carbon number	Composition (%)					Representative chemical structure
			Vinyl	Vinylidene	Di-sub (cis/ trans)	Tri-sub	Tetra-sub	
Arachis Oil		NA	NA	NA	NA	NA	NA	NA
Hex−1-ene	592–41–6	8	**> 95**	< 1	< 1	< 1	0	
Nonene, branched	97280–95–0	9	0	< 5	~30	**~65**	< 1	
Octadec−1-ene	112–88–9	18	~60	**~35**	~5	0	0	
Octadecene	27070–58–2	18	< 5	< 5	~60	~30	0	
Hydrocarbons, C12–30, olefin-rich, ethylene polymn. by product	68911–05–7	23.6	< 5	< 5	**~70**	~10	**~10**	

The highest concentration of any one structural element is marked in **bold**
NA: Not Applicable

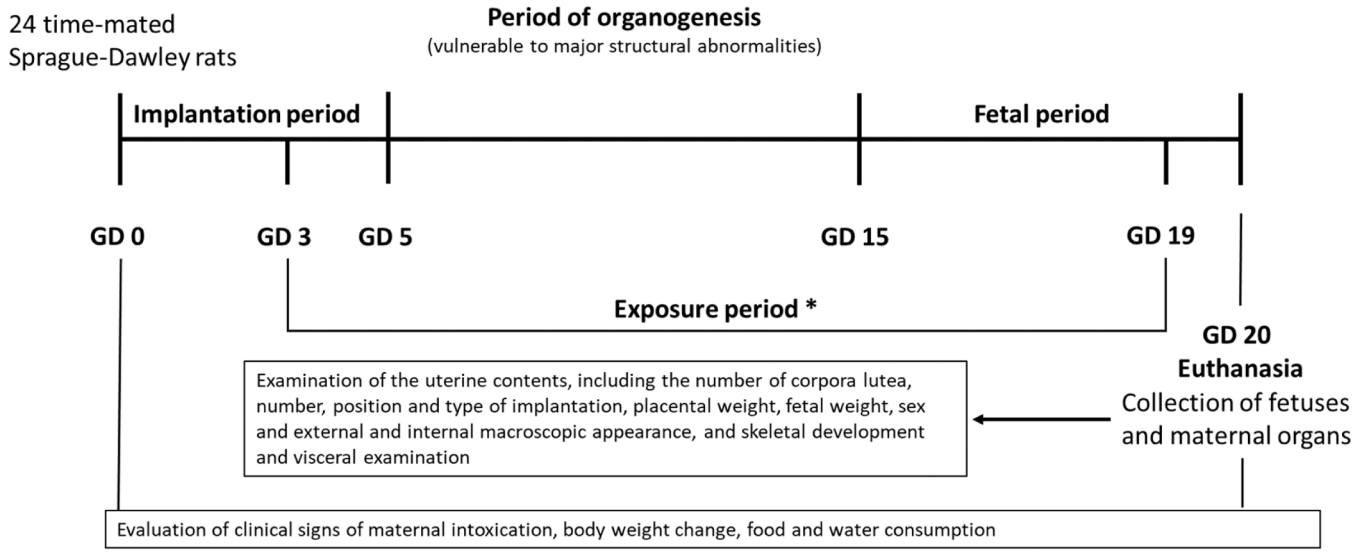


Fig. 1. OECD 414 rats experiment design [16]. GD = gestational day. * for Octadecene and Hydrocarbon, C12–30, olefins-rich, ethylene polymn. by product, the dosing period is between GD 5 and GD 19.

Body weights were recorded on Days 3 (prior to dosing) and on Days 4 (only for Hex-1-ene, Nonene, branched, and Octadec-1-ene), 5 (prior to dosing for Octadecene and Hydrocarbons C12–30, olefin-rich, ethylene polymn. by product), 8, 11, 14 and 17 of gestation. Body weights were also recorded for animals at terminal sacrifice (Day 20). Food consumption was recorded for each individual animal at Day 3, 5, 8, 11, 14, 17 and 20 of gestation. Water intake was observed daily by visual inspection of the water bottles for any overt changes.

On Day 20 of gestation, all animals were euthanized, followed by cervical dislocation. A comprehensive external and internal examination was conducted, noting any abnormalities. The ovaries and uteri of pregnant females were examined, recording the number of corpora lutea, intrauterine implantations, fetal sex, external appearance, fetal and placental weight, and gravid uterus weight. Non-pregnant uteri were tested to detect implantation evidence, categorizing implantations into Early Death, Late Death, and Dead Fetus. For litter assessment, parameters evaluated including pre-implantation loss, post-implantation, and sex ratio. In addition, fetuses were euthanized and divided into two groups for skeletal and soft tissue examinations. Alternate fetuses were fixed in Bouin's solution, examined for visceral anomalies, and stored in 10 % Buffered Formalin. The remaining fetuses were tagged, placed in 70 % IMS, eviscerated, stained with alizarin red S, and stored in 50 % glycerol for skeletal examination.

2.4. Statistical analysis

Data were first analyzed using Shapiro Wilk normality test and Bartlett's test for homogeneity of variance. Where there was no significance, parametric methodology was applied using one way analysis of variance and, if significant, Dunnett's multiple comparison test. Where statistical significance was observed, non-parametric methodology was applied using Kruskal-Wallis non-parametric analysis of variance; and, if significant, pairwise analysis of control values against treated values using the Mann-Whitney 'U' test. Due to the prevalence of non-normal

distributions for litter/fetal parameters, these data were routinely analyzed using non-parametric methodology. Fetal morphology parameters, including skeletal or visceral findings were analyzed by Kruskal Wallis and, if significant, Mann-Whitney 'U' test. For all analyses, differences were considered to be significant at the $p < 0.05$ level. GraphPad Prism (version 8.02) was used to generate figures.

3. Results

3.1. Maternal effects

3.1.1. Maternal mortality and clinical signs

Except for Nonene, branched, there were no unscheduled deaths upon any HO administration (Table 2). In Nonene, branched, one female treated with 1000 mg/kg bw/day was found dead on Day 16. This female was found to have a red stained ano-genital region and the bedding in the cage was also stained red. Another female from this treatment

Table 2
Summary of Endpoints conducted in OECD 414 rats studies for Hex-1-ene, Nonene, branched, Octadec-1-ene, Octadecene, and Hydrocarbons, C12-30, olefins-rich, ethylene polymn. by product.

Endpoints	Hex-1-ene	Nonene, branched	Octadec-1-ene	Octadecene	Hydrocarbons, C12-30, olefin-rich, ethylene polymn. by product
Dose level	0, 100, 300 or 1000 mg/kg bw/day	0, 100, 300 or 1000 mg/kg bw/day	0, 100, 300 or 1000 mg/kg bw/day	0, 100, 300 or 1000 mg/kg bw/day	0, 100, 300 or 1000 mg/kg bw/day
Mortality	No unscheduled deaths	One female from 1000 mg/kg bw/day dead on day 16 with a red stained ano-genital region. One female from 1000 mg/kg bw/day was killed in extremis on day 8 due to physical injury to one hind limb.	No unscheduled deaths	No unscheduled deaths	No unscheduled deaths
Clinical observations	No clinical signs of toxicity	Increased incidences of salivation in 20 out of 24 females treated with 1000 mg/kg bw/day.	One female treated with 100 mg/kg bw/day had a mass around the ano-genital region between Days 17 and 20.	One control female and one female treated with 300 mg/kg bw/day exhibited generalized fur loss. A further animal treated with 300 mg/kg bw/day also exhibited fur staining.	No clinical signs of toxicity
Water consumption	unaffected by treatment	unaffected by treatment	unaffected by treatment	unaffected by treatment	unaffected by treatment
Food consumption	unaffected by treatment	At 1000 mg/kg bw/day showed significantly lower food consumption during Days 3–8 of gestation	unaffected by treatment	unaffected by treatment	unaffected by treatment
Body weight and body weight gain	unaffected by treatment	At 1000 mg/kg bw/day, body weight gain was significantly lower than control on Days 5, 8 and 14.	unaffected by treatment	unaffected by treatment	unaffected by treatment
Necropsy	No macroscopic abnormalities were apparent for treated females at Day 20 of gestation.	No macroscopic abnormalities were apparent for treated females at Day 20 of gestation.	No macroscopic abnormalities were apparent for treated females at Day 20 of gestation.	No macroscopic abnormalities were apparent for treated females at Day 20 of gestation.	No macroscopic abnormalities were apparent for treated females at Day 20 of gestation.
Litter data, littler placental and fetal weights	The number of implantations, subsequent embryofetal survival and litter size, sex ratio and mean fetal, litter and placental weights on Day 20 of gestation were unaffected by maternal treatment at 100, 300 and 1000 mg/kg bw/day. A statistically significant reduction in pre-implantation loss was observed in females treated with 300 and 1000 mg/kg bw/day	The number of implantations, subsequent embryofetal survival and litter size, sex ratio and mean fetal, litter and placental weights on Day 20 of gestation were unaffected by maternal treatment at 100, 300 and 1000 mg/kg bw/day.	The number of implantations, subsequent embryofetal survival and litter size, sex ratio and mean fetal, litter and placental weights on Day 20 of gestation were unaffected by maternal treatment at 100, 300 and 1000 mg/kg bw/day.	The number of implantations, subsequent embryofetal survival and litter size, sex ratio and mean fetal, litter and placental weights on Day 20 of gestation were unaffected by maternal treatment at 100, 300 and 1000 mg/kg bw/day.	The number of implantations, subsequent embryofetal survival and litter size, sex ratio and mean fetal, litter and placental weights on Day 20 of gestation were unaffected by maternal treatment at 100, 300 and 1000 mg/kg bw/day.
Fetal examination	Females from all treatment groups showed a statistically significant reduction in the percent of fetuses showing a dumb bell-shaped thoracic centrum.	Neither the type, incidence or distribution of findings observed during external examination of the fetuses at necropsy on Day 20 of gestation or subsequent detailed visceral and skeletal examinations indicated any obvious effect of maternal treatment on fetal development.	Females treated with 1000 and 300 mg/kg bw/day showed a statistically significant reduction in the percent of fetuses showing incomplete ossification of the frontal bone.	Neither the type, incidence or distribution of findings observed during external examination of the fetuses at necropsy on Day 20 of gestation or subsequent detailed visceral and skeletal examinations indicated any obvious effect of maternal treatment on fetal development.	Neither the type, incidence or distribution of findings observed during external examination of the fetuses at necropsy on Day 20 of gestation or subsequent detailed visceral and skeletal examinations indicated any obvious effect of maternal treatment on fetal development.

group was killed in extremis on Day 8 due to a physical injury to one hind limb.

There were no clinical signs of toxicity detected in animals dosed with Hex-1-ene and Hydrocarbons, C12–30, olefin-rich, ethylene polymn. by product (Table 2). Nonene, branched increased the incidence of salivation in a number of females treated with 1000 mg/kg bw/day between Days 14 and 19. One female treated with 100 mg/kg bw/day Octadec-1-ene had a mass around the ano-genital region between Days 17 and 20. One control and one female treated with 300 mg/kg bw/day Octadecene showed generalized fur loss, and one female treated with 300 mg/kg bw/day Octadecene exhibited fur staining.

3.1.2. Maternal food and water consumption

For all test materials, the water consumption of all dose groups was unaffected during the treatment period (Table 2). Except for Nonene, branched, all test materials also showed no effects on food consumption during gestation at any dose level (Fig. 2). At 1000 mg/kg bw/day of Nonene, branched, food consumption appeared lower than control during Days 3–8 of gestation, with differences attaining statistical significance (18.8 % lower at day 3–5, $p < 0.001$, 10.9 % lower at day 5 – 8, $p < 0.05$, respectively). Recovery was evident thereafter. In addition, no such effects were apparent with treatment at 100 and 300 mg/kg bw/day.

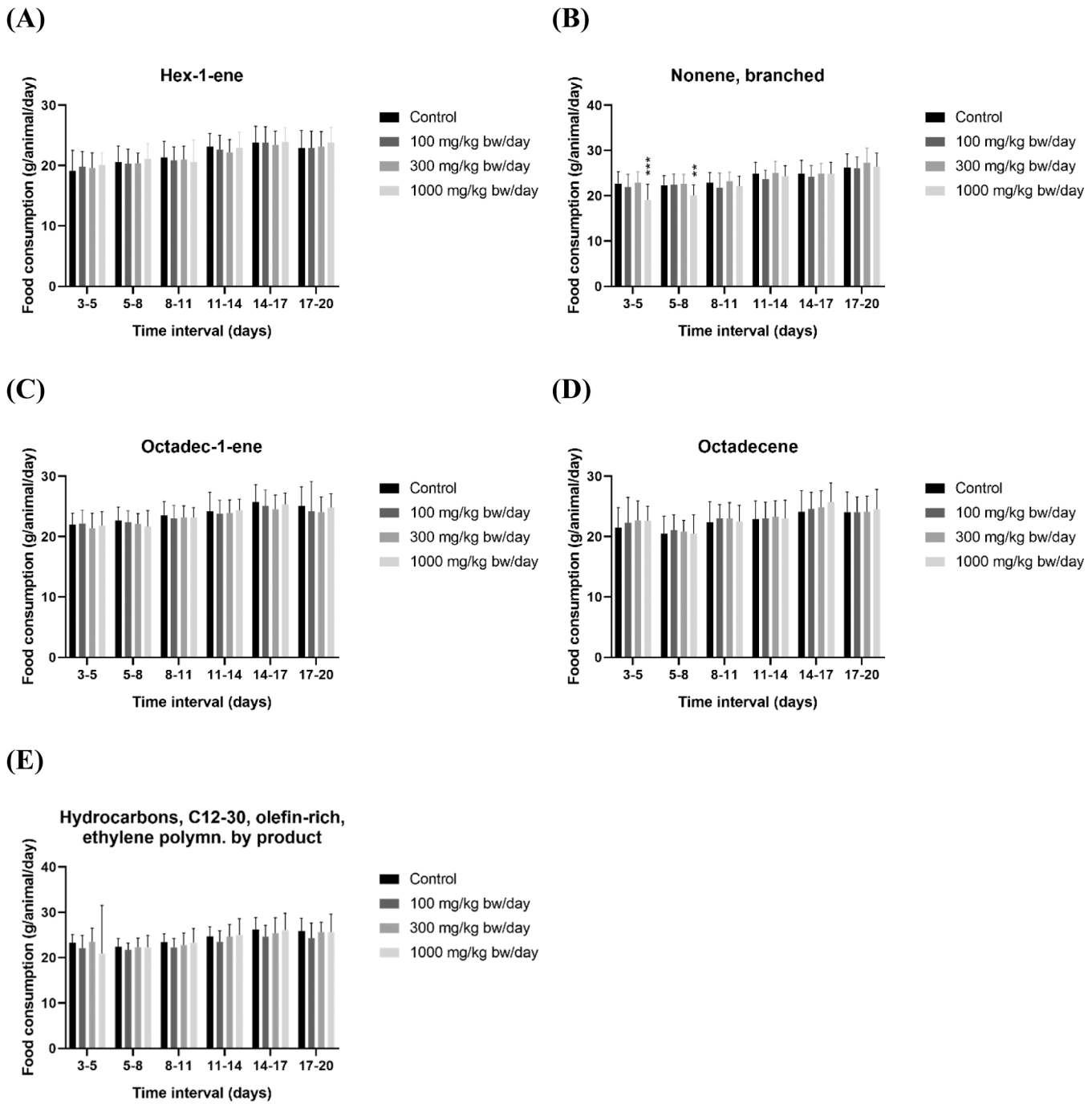


Fig. 2. Food consumption of pregnant rats. (A) Hex-1-ene, (B) Nonene, branched, (C) Octadec-1-ene, (D) Octadecene, and (E) Hydrocarbons, C12–30, olefin-rich, ethylene polymn. by product. Significantly difference from control value (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.1.3. Maternal body weight and body weight gain

Except for Nonene, branched, the body weight and body weight gain, including adjustment for the contribution of the gravid uterus, were unaffected by all test materials at 100, 300 or 1000 mg/kg bw/day (Figs. 3 and 4, and Table 3). For females treated with 1000 mg/kg bw/day of Nonene, branched, body weight gain between Days 3 and 8 was lower than control, however, differences only attained statistical significance between Days 5–8. Body weight gain for these females during each measured period from Day 11 onwards was however comparable to controls. The lower initial body weight gain resulted in differences in cumulative body weight gain throughout gestation and attaining statistical significance on Days 5 (51.4 % lower, $p < 0.05$), 8 (43.3 % lower, $p < 0.001$) and 14 (15.8 % lower, $p < 0.05$). The lower overall body weight gain remained statistically significant (22.5 % lower, $p < 0.05$), even when adjusted for the contribution of the gravid uterus. No such effects were observed by treatment at 100 and 300 mg/kg bw/day.

3.1.4. Maternal necropsy

On necropsy, there were no treatment-related gross changes/macroscopic abnormalities noticed in the treated females for any of the tested HO (Table 2).

The female treated with 1000 mg/kg bw/day Nonene, branched that was found dead on Day 16 had red staining around the vagina and the right horn of the uterus contained red colored contents. In addition, the female that was killed in extremis on Day 8 had a swollen and broken right hind limb.

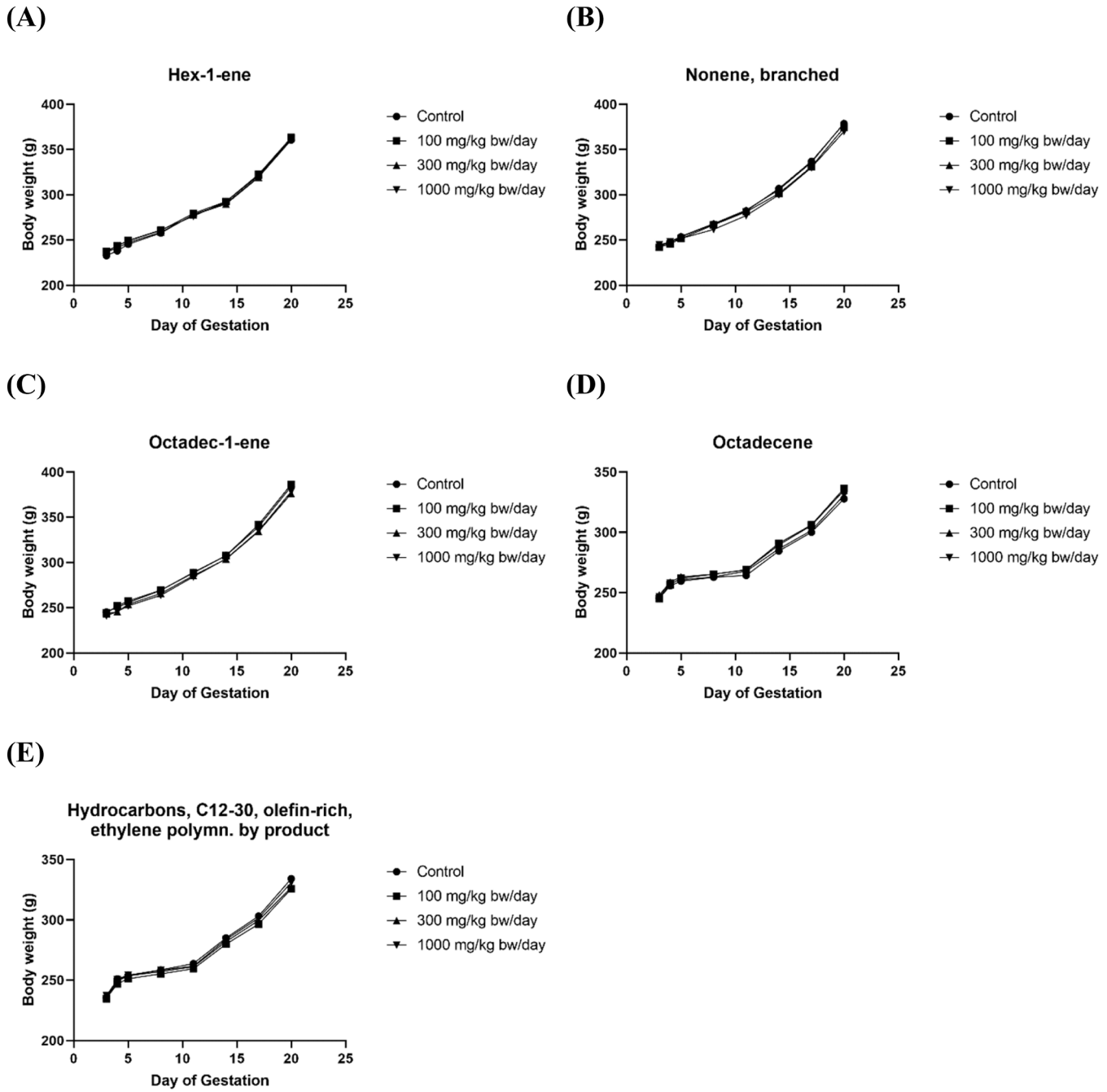


Fig. 3. Body weight of pregnant rats. (A) Hex-1-ene, (B) Nonene, branched, (C) Octadec-1-ene, (D) Octadecene, and (E) Hydrocarbons, C12–30, olefin-rich, ethylene polymn. by product. Significantly difference from control value (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

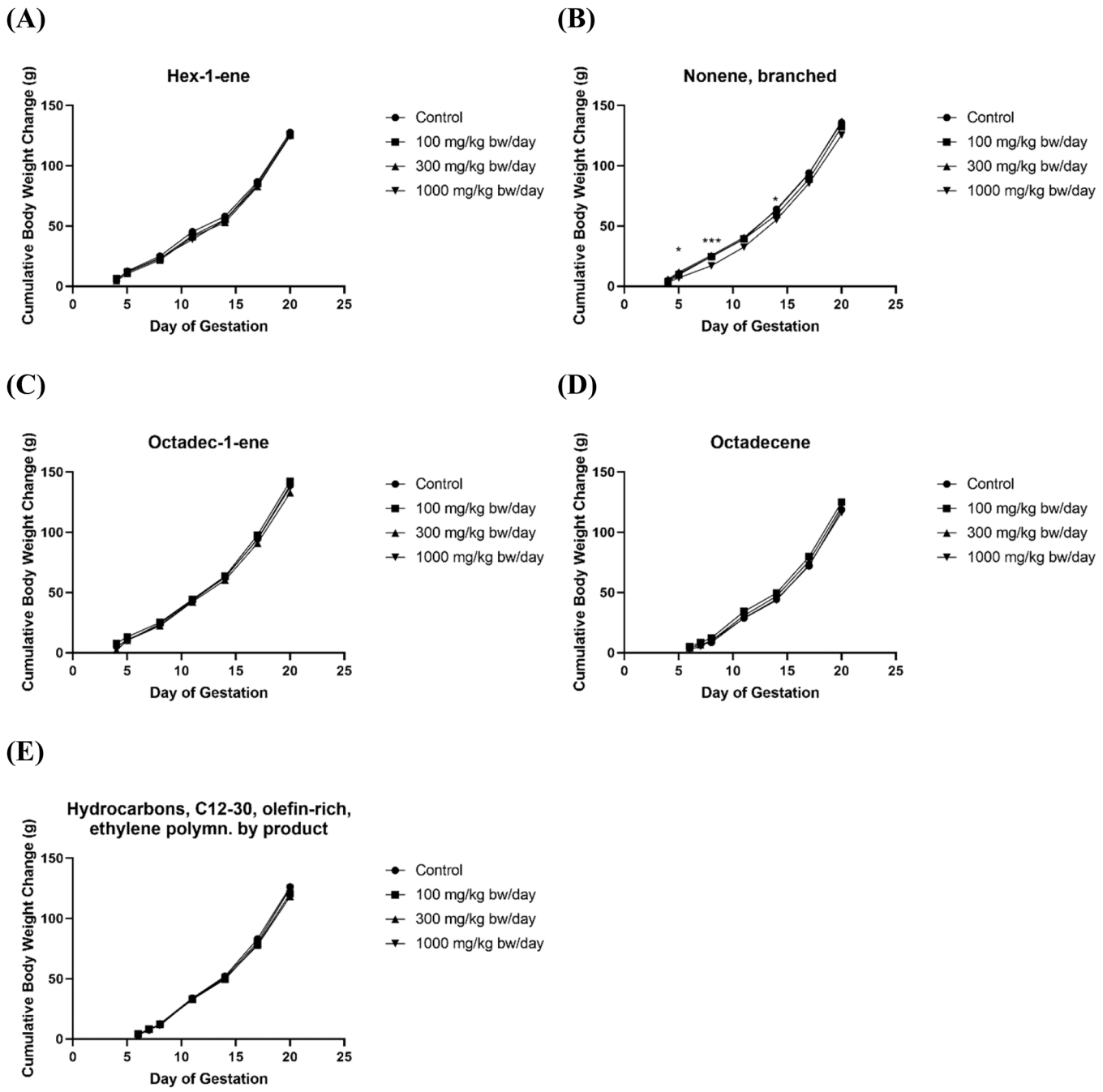


Fig. 4. Body weight gain of pregnant rats. (A) Hex-1-ene, (B) Nonene, branched, (C) Octadec-1-ene, (D) Octadecene, and (E) Hydrocarbons, C12–30, olefin-rich, ethylene polymn. by product. Significantly difference from control value (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.2. Effects on fetuses

3.2.1. Litter data, litter placental and fetal weights

For all test materials, the number of implantations, subsequent embryofetal survival and litter size, sex ratio and mean fetal, litter and placental weights on Day 20 of gestation were unaffected by maternal treatment at 100, 300 and 1000 mg/kg bw/day (Appendix Table 1 - Table 5). Interestingly, females treated with 300 and 1000 mg/kg/day of Hex-1-ene experienced a statistically significant lower pre-implantation loss compared to controls.

3.2.2. Fetal examination

For Nonene, branched, Octadecene and Hydrocarbons, C12–30, olefin-rich, ethylene polymn. by product, neither the type, incidence nor distribution of findings observed during external examination of the fetuses at necropsy on Day 20 of gestation and subsequent detailed visceral and skeletal examination indicated any adverse effect of maternal treatment on fetal development (Table 2).

Fetuses from Hex-1-ene and Octadec-1-ene treated dams displayed advanced maturation compared to controls. For instance, females from all treatment groups of Hex-1-ene showed a statistically significant reduction in the percent of fetuses showing a dumb bell-shaped thoracic centrum (Appendix Table 6) and females treated with 1000 and 300 mg/kg bw/day of Octadec-1-ene showed a statistically significant reduction in the percent of fetuses showing incomplete ossification of the frontal bone (Appendix Table 7).

Table 3
Group mean gravid uterus weight and adjusted body weight and body weight change.

Substances	Dose level (mg/kg bw/day)	Body weight (g) on days of Gestation		Body weight changes during the days of Gestation (Day 3–20)	Gravid uterus weight (g)	Adjusted body weight (g) at day 20	Adjusted body weight change (g) day 3 vs day 20
		Day 3	Day 20				
Hex−1-ene	Control	232.8 ± 25.7	360.5 ± 38.2	127.7 ± 19.1	80.6 ± 12.0	279.8 ± 30.7	47.0 ± 13.4
	100	237.3 ± 18.5	363.2 ± 31.0	125.9 ± 17.9	81.3 ± 12.6	281.9 ± 23.3	44.5 ± 12.4
	300	236.7 ± 16.8	362.0 ± 25.1	125.3 ± 18.3	82.6 ± 12.5	279.4 ± 21.0	42.7 ± 12.1
	1000	237.6 ± 20.2	362.1 ± 33.7	124.6 ± 18.0	77.2 ± 13.8	285.0 ± 26.0	47.4 ± 11.0
Nonene, branched	Control	243.0 ± 19.4	378.8 ± 33.5	135.8 ± 23.1	81.2 ± 17.7	297.5 ± 23.6	54.5 ± 11.7
	100	242.0 ± 17.1	374.3 ± 22.3	132.3 ± 17.5	82.7 ± 12.8	291.6 ± 20.1	49.5 ± 13.4
	300	242.4 ± 13.3	379.2 ± 28.5	136.8 ± 20.7	81.5 ± 15.1	297.7 ± 20.7	55.3 ± 15.2
	1000	244.7 ± 14.3	370.1 ± 27.9	125.5 ± 17.2	80.9 ± 13.0	289.2 ± 22.3	44.5 ± 11.8 *
Octadec−1-ene	Control	245.2 ± 15.7	384.1 ± 32.2	138.9 ± 22.3	82.9 ± 17.2	301.2 ± 21.5	56.0 ± 11.9
	100	244.2 ± 15.6	386.4 ± 21.8	142.2 ± 16.9	88.3 ± 8.2	298.1 ± 19.5	53.9 ± 15.5
	300	243.4 ± 11.8	376.0 ± 26.8	132.7 ± 22.5	81.6 ± 17.5	294.5 ± 20.9	51.1 ± 14.1
	1000	241.1 ± 17.1	378.4 ± 19.1	137.3 ± 15.5	84.0 ± 7.9	294.3 ± 17.6	53.2 ± 14.1
Octadecene#	Control	255.7 ± 30.7	374.5 ± 48.6	118.8 ± 21.3	83.7 ± 12.8	290.8 ± 39.0	35.1 ± 12.5
	100	256.7 ± 22.4	381.8 ± 31.2	125.0 ± 18.5	86.9 ± 11.1	294.9 ± 26.7	38.2 ± 13.2
	300	258.8 ± 20.4	380.0 ± 24.7	121.2 ± 14.1	87.8 ± 8.9	292.2 ± 22.8	33.4 ± 10.6
	1000	257.8 ± 24.6	374.1 ± 39.6	116.3 ± 19.9	82.0 ± 15.8	292.2 ± 31.6	34.3 ± 13.0
Hydrocarbons, C12–30, olefin-rich, ethylene polymn. by product#	Control	251.2 ± 22.1	377.4 ± 32.3	126.2 ± 15.0	83.4 ± 8.5	294.0 ± 28.4	42.8 ± 12.0
	100	247.1 ± 18.4	367.9 ± 32.3	120.7 ± 17.3	82.5 ± 11.2	285.4 ± 26.2	38.3 ± 10.8
	300	249.3 ± 18.9	367.6 ± 31.7	118.3 ± 19.3	74.5 ± 20.4	293.1 ± 22.5	43.8 ± 12.5
	1000	250.5 ± 20.6	375.4 ± 33.5	124.9 ± 17.4	83.4 ± 11.6	292.0 ± 29.1	41.5 ± 13.0

The results are presented as the mean±SD.
*$p < 0.05$; * *$p < 0.01$; * **$p < 0.001$; significant different from vehicle control.
body weight parameters were measured from day 5.

4. Discussions

HO are the building blocks for polymers and oligomers used in plastics, lubricants, detergents, solvents, etc [2,3]. However, little is known about the potential teratogenic effects of HO since only screening-level information (i.e. OECD 421/422) is publicly available. In addition, in our previous OECD 422 study, Nonene, branched raised concerns due to the observed reduction in litter size and litter weights at the highest dose level (1000 mg/kg bw/day), because if the exposure of mother and embryo to a teratogenic agent during the development stage (5th to 15th day of gestation) affects the development of the embryo, the teratogen may continue affecting the functions and growth of organs of fetuses beyond the 15th day of gestation [17–19]. Therefore, our current study was conducted to investigate the possible prenatal developmental toxicity effects of HO in pregnant Sprague-Dawley rats during the gestation according to the OECD guideline No. 414 [16].

Two adult females treated with Nonene, branched at 1000 mg/kg bw/day were found dead. The first female had red staining around the vagina and the right horn of the uterus contained red colored contents on Day 16, and this death was most likely due to the intra-uterine total litter loss that was evident. In the absence of any other significant effects in the remaining females or on fetus survival, it was considered unrelated to treatment. The second female that was killed *in extremis* had a swollen and broken right hind limb. This was due to a physical injury and unrelated to treatment. The increased salivation evident with treatment at 1000 mg/kg bw/day of Nonene, branched was also observed in our previous OECD 422 and OECD 408 studies. This effect is attributed to the unpalatable or mildly irritating nature of the test material when administered via oral gavage, and it is considered to have no toxicological significance [10,11]. Additionally, with prolonged dosing, irritative effects on the stomach may occur. In the OECD 408 study, acanthosis of the forestomach was observed in male rats at 500 mg/kg bw/day [11]. In the OECD 422 study, epithelial hyperplasia in the forestomach was reported in both sexes at 1000 mg/kg bw/day and in males at 300 mg/kg bw/day [10].

Increases in body weights and food consumption as the animals grow, is a simple yet sensitive index of adverse effects, where decreased body weight gains along with a decrease in food consumption is considered adverse [20–22]. Generally, a decrease of more than 10 % in body weight gain relative to control is considered an adverse effect when assessing toxicity [21]. The oral administration of Nonene, branched to pregnant rats during organogenesis at dose levels of 1000 mg/kg/day was associated with lower initial body weight gain and food intake in females between Days 3 and 8 of gestation. Subsequently, cumulative body weight gain throughout gestation and overall body weight gain when adjusted for the contribution of the gravid uterus were lower.

However, body weight gain for these females during each measured period from Day 11 onwards was comparable to controls, therefore suggesting that the effect on body weight was an initial response to treatment and did not represent an overall adverse effect of treatment.

Likewise, across five current studies investigating the fetal developmental toxicity of various HOs with doses up to 1000 mg/kg bw/day consistently showed no significant impact on key parameters such as the number of implantations, embryofetal survival, litter size, sex ratio, and mean fetal, litter, and placental weights on Day 20 of gestation and subsequent detailed visceral and skeletal examination. While some fetal parameters showed statistically significant changes following exposure to Hex-1-ene and Octadec-1-ene (see Appendix Tables 6 and 7), these findings were non-adverse, did not demonstrate dose-related trends or clusters of developmental variations, and were therefore considered incidental, lacking biological or toxicological relevance. For instance, Hex-1-ene reduced dumbbell-shaped thoracic centra and Octadec-1-ene reduced fetuses with incomplete ossification of the frontal bone. Both observations are non-adverse because the treated fetuses are more developmentally advanced compared to controls.

5. Conclusions

Based on the observations and analyses under the conditions of the present studies, all five HO (i.e. Hex-1-ene, Nonene, branched, Octadec-1-ene, Octadecene, and Hydrocarbons, C12–30, olefin-rich, ethylene polymn. by product) had no toxicological effects on pregnant rats or their embryos and fetuses, and the no observed adverse effect level (NOAEL) for all test materials were estimated to be 1000 mg/kg bw/day for both pregnant rats (maternal toxicity) and their embryos and fetuses (developmental toxicity).

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Juan-Carlos Carrillo: Writing – review & editing, Formal analysis, Data curation. **Jamie Dunn:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Hua Shen:** Formal analysis, Data curation. **Sophie Jia:** Writing – review & editing, Investigation, Formal analysis. **An R Van Rompay:** Writing – review & editing, Formal analysis. **Fabienne Hubert:** Formal analysis, Data curation, Conceptualization. **Peter J Boogaard:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Quan Shi:** Writing – original draft, Formal analysis. **Michael G Penman:** Writing – review & editing, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Quan Shi reports a relationship with Shell Global Solutions International BV that includes: employment. Juan-Carlos Carrillo reports a relationship with Shell Global Solutions International BV that includes: employment. Hua Shen reports a relationship with Shell Oil Company that includes: employment. Michael G Penman reports a relationship with Penman Consulting that includes: employment. Jamie Dunn reports a relationship with Penman Consulting that includes: employment. An R Van Rompay reports a relationship with Penman Consulting that includes: employment. Sophie Jia reports a relationship with Chevron Phillips Chemical Co LP that includes: employment. Fabienne Hubert reports a relationship with INEOS Olefins and Polymers Europe that includes: employment. Co-author Peter J Boogaard previously employed by Shell Global Solutions International B.V. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.reprotox.2024.108756.

Data availability

Data will be made available on request.

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