Original Article

Developmental Toxicity Studies with Atrazine and its Major Metabolites in Rats and Rabbits

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Atrazine (ATR), hydroxyatrazine (OH-ATR), and the three chloro metabolites of ATR (deethylatrazine [DEA], deisopropylatrazine [DIA], diaminochlorotriazine [DACT]) were evaluated for developmental effects in rats and rabbits. Three developmental toxicity studies were conducted on ATR in rats (two studies) and rabbits and a developmental toxicity study was conducted in rats for each of the four ATR metabolites DEA, DIA, DACT, and OH-ATZ. ATR administration by gavage to pregnant rats and rabbits from implantation (gestation day [GD] 6 in rat, GD 7 in rabbit) through closure of the palate (GD 15 in rat and GD 19 in rabbit) did not statistically significantly alter the incidence of developmental abnormalities or malformations at dose levels up to 100 (rat) or 75 (rabbit) mg/kg bw/day. There were no effects on developmental toxicity parameters for DEA, DIA, DACT, or OH-ATR at oral dose levels up to 100, 100, 150, or 125 mg/kg bw/day, respectively, with the exception of reductions in fetal body weight by DACT and OH-ATR in the presence of decreased maternal body weight gain. ATR did not adversely affect developmental end points in a two-generation study conducted in rats exposed to dose levels up to 500 ppm (38.7 mg/kg/day) in the diet. The 500-ppm dose level resulted in significantly reduced maternal body weight gain. Overall, data show that neither ATR nor its metabolites statistically significantly affected rat or rabbit embryo-fetal development even at dose levels producing maternal toxicity. *Birth Defects Res (Part B)* 00:1–16, 2014. © 2014 Wiley Periodicals, Inc.

Key words: ***atrazine; hydroxyatrazine; deethylatrazine; deisopropylatrazine; diaminochlorotriazine; developmental toxicity; teratology***

INTRODUCTION

Atrazine (ATR) is a chlorotriazine herbicide that inhibits photosynthesis in plants by preventing electron transfer at the reducing site of chloroplast complex II. ATR is used to control broadleaf weeds predominantly in corn, sorghum, and sugar cane. The metabolic pathways for ATR are shown in Figure 1. ATR is rapidly metabolized by animals to deethylatrazine (DEA), deisopropylatrazine (DIA), and diaminochlorotriazine (DACT). In workers exposed to ATR, 80% of the estimated dose appears in the urine as DACT, 10% as DIA, and 8% as DEA (Catenacci et al., 1993). Hydroxyatrazine (OH-ATR) is a major plant metabolite and may be found in surface soils and sediments.

Epidemiology studies evaluating the association between agricultural practices including the use of ATR and the prevalence of birth defects or smallness for gestational age have been reviewed by Goodman et al. (2014). To assist in the interpretation of potential human developmental effects of ATR and its metabolites, this communication presents results from three developmental toxicity studies on ATR (two in the rat and one in the rabbit) and one developmental toxicity study conducted in rats for OH-ATZ, DEA, DIA, and DACT. The results from two multigeneration reproduction studies conducted on ATR are presented in a companion paper (DeSesso et al, 2014). In a second companion paper (Foradori et al., 2014), the effects of 4 days of ATZ exposure (gavage or diet) on the preovulatory luteinizing hormone surge and subsequent ovulation in females Sprague–Dawley and Long Evans rats are described. All studies, except those initiated before 1966, were conducted in toxicology laboratories under Good Laboratory Practices guidelines and adhered to contemporary animal welfare regulations. Quality assurance audited final reports were submitted to regulatory agencies around the world, including the U.S. Environmental Protection Agency. The first rat developmental toxicity

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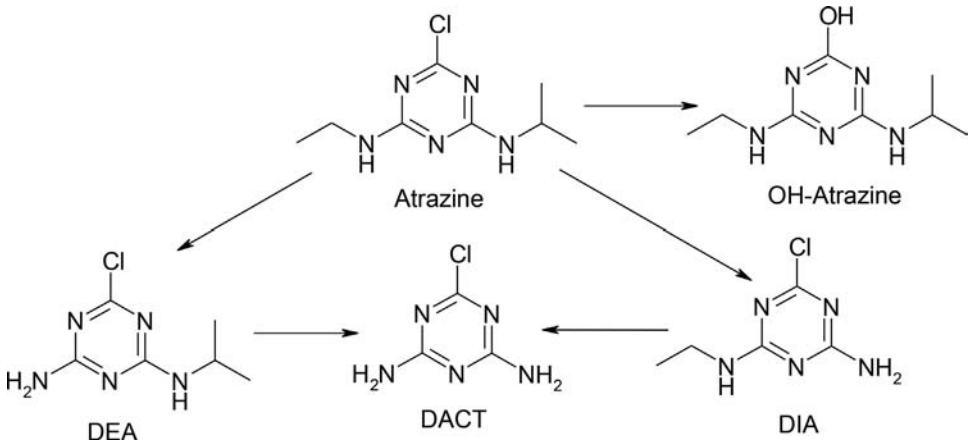

Fig. 1. In animals, atrazine is mono-dealkylated to deisopropylatrazine (DIA) or deethylatrazine (DEA) then further dealkylated to form the di-dealkylated metabolite, diaminochlorotriazine (DACT); the most prominent plant metabolite is hydroxyatrazine (OH-atrazine).

and rabbit developmental toxicity study were previously published (Infurna et al., 1988). The remaining studies have not been previously published.

METHODS

Design features of the multigeneration and prenatal studies are summarized in Table 1. Except for the three-generation study, which was performed in 1966, these studies were performed between 1984 and 1992. Details of the multigeneration studies are provided in the companion paper (DeSesso et al., 2014).

Developmental Toxicity Studies of ATR in Rats

There were two rat developmental toxicity studies of ATR that differed with respect to the dose levels that were administered and the method of fetal evaluation. In the first study, completed in 1984 and published in 1988 (Infurna et al., 1988), ATR was administered at doses of 0, 10, 70, or 700 mg/kg bw/day to 27 mated females per dose group; in the second study, completed in 1989, ATR was administered at doses of 0, 5, 25, or 100 mg/kg bw/day to 24 mated females per dose group. The rationale for dose selection was not given in the study reports. The day of finding evidence of mating was designated gestation day (GD) 0. In both studies, pregnant dams were treated by gavage on GD 6 to 15 and necropsied on GD 20. Ovaries were examined and corpora lutea counted. Fetuses were weighed and examined externally. Two-thirds (1984 study) or one-half (1989 study) of the fetuses in each litter were stored in 70% ethanol for skeletal examination after maceration, alizarin red S staining, and clearing while the remaining fetuses in each litter were examined viscerally by the freehand sectioning method of Wilson (1965) after fixation in Bouin's solution. A maceration step before clearing was not described in either study report but is assumed based on usual practices of the time period. There were minor differences between the two studies.

Developmental Toxicity Studies of ATR in Rabbits

Infurna et al. (1988) administered ATR by gavage to pregnant rabbits on GD 7 to 19 at dose levels of 0, 1, 5, or 75 mg/kg bw/day. The day of artificial insemination was designated as GD 0. Does were observed daily for changes in appearance and behavior and were weighed on GD 0, 7, 14, 19, 21, 25, and 29. Food consumption was measured daily. Does were necropsied on GD 29 after carbon dioxide asphyxiation. Ovaries were examined and corpora lutea counted. Uteri and contents were weighed. Fetuses were examined for external abnormalities and each fetus was examined by fresh visceral dissection (Stuckhardt and Poppe, 1984) followed by preparation for skeletal evaluations by the alizarin red S method.

Developmental Toxicity Studies of ATR Metabolites in Rats

The metabolites DIA, DEA, DACT, and OH-ATR were evaluated in rat developmental toxicity studies of similar design (Table 1). Dose levels for each test material are presented in Table 1 and were based on preliminary dose range-finding studies. Dams were observed daily for change in appearance or behavior and were weighed daily. For DIA and DEA, food consumption was recorded on GD 6, 11, 16, and 21 (evidence of mating = GD 0), and a daily mean was calculated from average consumption during each 5-day period. For DACT and OH-ATR, food consumption and weight were recorded on GD 0, 6, 8, 12, 16, and 20. In all studies, dams were killed on GD 21 by carbon dioxide asphyxiation and fetuses evaluated for external abnormalities. About half the fetuses in each litter were fixed in Bouin's fluid for visceral examination by freehand sectioning, and the remainder were stored in 70% ethanol, macerated in potassium hydroxide, cleared in graded glycerin solutions, and stained with alizarin red S for skeletal examination.

Statistical Analysis

Parametric data are presented as means ± SD. Parametric data with homogeneous variances by the Bartlett

Table 1
Details of Study Designs

Study, year	Species, strain, *n*	Environment	Food and water	Dosing
		Atrazine		
Rat three-generation, 1966	Albino rats from Charles River, 10 males and 20 females per dose group	Individual cages	Purina Certified Rodent Chow and water ad libitum	Dietary atrazine at 0, 50, or 100 ppm; food consumption not reported
Rat two-generation, 1987	CR(CD) VAF/Plus rats, 30 males and 30 females per dose group	Individual, solid bottom cage with wood shavings; 23 ± 3°C, 50 ± 20% relative humidity, 14/10 light/dark cycle	Purina no. 5002 Certified Rodent Chow and water ad libitum	Dietary atrazine at 0, 10, 50, or 500 ppm; estimated dose levels 0, 0.73, 3.64, 38.7 mg/kg bw/day
Rat developmental toxicity, 1984[a]	Crl. COBS™ CD™ (SD) (BR) rats from Charles River, 27 virgin females per dose group were mated	Individual (except during mating), solid bottom cage with wood shavings; 23 ± 3°C, 50 ± 20% relative humidity, 14/10 light/dark cycle	Purina no. 5502 Certified Rodent Chow and water ad libitum	Atrazine in 3% aqueous corn starch containing 0.5% Tween-80 by gavage at 0, 10, 70, or 700 mg/kg bw/day, 10 ml/kg bw/day, GD 6–15
Rat developmental toxicity, 1989	Crl. COBS™ CD™ (SD) (BR) rats from Charles River, 24 virgin females per dose group were mated	Individual (except during mating), solid bottom cage with wood shavings; 23 ± 3°C, 50 ± 20% relative humidity, 14/10 light/dark cycle	Purina no. 5502 Certified Rodent Chow and water ad libitum	Atrazine in 3% aqueous corn starch containing 0.5% Tween-80 by gavage at 0, 5, 25, or 100 mg/kg bw/day, 10 ml/kg bw/day, GD 6–15
Rabbit developmental toxicity, 1984[a]	New Zealand white rabbits from HARE Rabbits for Research; 19 inseminated virgin females per dose group	Individual meshed bottom-less cages, 18 ± 3°C, 50 ± 20% relative humidity, 14/10 light/dark cycle	Purina Certified Rabbit Chow and water ad libitum	Atrazine in 3% aqueous corn starch containing 0.5% Tween-80 by gavage at 0, 1, 5, or 75 mg/kg bw/day, 5 ml/kg bw/day, GD 7–19
		Deisopropylatrazine (DIA)		
Rat developmental toxicity, 1992	Tif:RAI f (SPF) stock rats maintained by CIBA-GEIGY, Switzerland; 24 mated nulliparous rats per dose group	Individual solid-bottom cages with granulated wood bedding; 22 ± 3°C, 50 ± 20% relative humidity, 12/12 light/dark cycle	Nafag no. 890 diet and water ad libitum	DIA in 3% aqueous corn starch by gavage at 0, 5, 25, or 100 mg/kg bw/day, 10 ml/kg bw/day, GD 6–15
		Deethylatrazine (DEA)		
Rat developmental toxicity, 1992	Tif:RAI f (SPF) stock rats maintained by CIBA-GEIGY, Switzerland; 24 mated nulliparous rats per dose group	Individual solid-bottom cages with granulated wood bedding; 22 ± 3°C, 50 ± 20% relative humidity, 12/12 light/dark cycle	Nafag no. 890 diet and water ad libitum	DEA in 3% aqueous corn starch by gavage at 0, 5, 25, or 100 mg/kg bw/day, 10 ml/kg bw/day, GD 6–15
		Diaminochlorotriazine (DACT)		
Rat developmental toxicity, 1989	Crl:COBS CD (SD) BR rats from Charles River, 26 mated females per dose group	Individual solid-bottom cages with granulated wood bedding; 23 ± 3°C, 50 ± 20% relative humidity, 14/10 light/dark cycle	Purina no. 5002 Certified Rodent Chow and water ad libitum	DACT in 3% aqueous corn starch by gavage at 0, 2.5, 25, 75, or 150 mg/kg, bw/day, 10 ml/kg bw/day, GD 6–15
		Hydroxyatrazine (OH-ATR)		
Rat developmental toxicity, 1989	Crl:COBS CD (SD) BR rats from Charles River, 30 mated females per dose group	Individual solid-bottom cages with hardwood chip bedding; 23 ± 3°C, 50 ± 20% relative humidity, 14/10 light/dark cycle	Purina no. 5002 Certified Rodent Chow and water ad libitum	OH-ATR in 3% aqueous corn starch containing 0.5% Tween-80 by gavage at 0, 5, 25, or 120 mg/kg bw/day, 10 ml/kg bw/day, GD 6–15

The developmental studies included an evaluation of maternal body weight and weight gain, feed consumption, corpora lutea number, implantation number, preimplantation loss, resorptions, postimplantation loss, litter size, sex ratio, fetal body weights, external alterations, visceral alterations, and skeletal alterations.
[a]Published as Infurna et al. (1988).

Table 2
1984 Rat Developmental Toxicity Study of Atrazine: Fertility and Developmental End Points[a]

End point	Atrazine dose (mg/kg bw/day)			
	0	10	70	700
Total number of mated females	27	27	27	27
Total number of pregnant females	24	23	25	26
Percent pregnant females	88.9	85.2	92.6	96.3
Percent maternal mortality	0	0	0	78*
Percent of maternal food consumption (GD 6–15)	100	101	95	61*
Maternal body weight gain in gram (GD 6–20)[b]	33	32	26	−54
Mean no. of corpora lutea ± SD	15.9 ± 2.5	15.6 ± 2.2	16.4 ± 1.9	16.0 ± 1.7
Mean no. of implantation sites ± SD	13.0 ± 4.8	14.6 ± 3.4	14.9 ± 3.1	12.8 ± 4.4
Number of litters examined	23	23	25	5[c]
Mean no. of total resorptions ± SD	0.83 ± 0.92	0.91 ± 0.90	0.92 ± 1.15	1.33 ± 2.4
Mean no. of dead fetuses per litter	0	0	0	0.3
Mean no. of live fetuses per litter ± SD	12.7 ± 3.9	13.7 ± 3.3	14.0 ± 3.0	11.2 ± 6.0
Preimplantation loss (%)	19.7	6.8	9.0	21.2
Postimplantation loss (%)	9.7	5.9	6.1	20.9
Sex ratio (% males)	51.7	50.0	53.9	46.3
Mean fetal body weight (g) ± SD, males	3.4 ± 0.21	3.6 ± 0.47	3.4 ± 0.34	1.9 ± 0.45*
Females	3.3 ± 0.20	3.4 ± 0.46	3.2 ± 0.38	1.8 ± 0.43*

[a]Published as Infurna et al. (1988).
[b]Terminal body weight on gestation day 20 minus uterus and conceptus weight.
[c]Only five litters were examined in the high-dose group due to maternal mortality.
*Different from the control group at $p \leq 0.05$.

test were analyzed using analysis of variance followed by the Dunnett *t*-test to evaluate differences in treated group means compared to the control group. The Dunn test based on rank sums was used if variances were not homogeneous. The litter was considered the statistical unit. For malformations within litters, the Mantel trend test was used across dose groups. Categorical data were analyzed using chi-square or Fisher's exact test. A value of $p < 0.05$ was accepted as statistically significant.

RESULTS

Multigeneration Studies of ATR in Rats

There were no treatment-related developmental abnormalities in the three- and two-generation studies. Details of the study results are given in the companion paper (DeSesso et al., 2014).

Developmental Toxicity Studies of ATR in Rats

Of the two rat developmental toxicity studies of ATR, the 1984 study (Infurna et al., 1988) included a 700 mg/kg bw/day dose level, which exceeded the maximum tolerated dose (MTD) as evidenced by a significant 39% reduction in food consumption (Table 2), 54 g mean body weight loss, increased clinical signs, and deaths of 21 of 27 dams by GD 20. A control female was found dead on GD 3, before dosing began, and was replaced. All other dams survived and were killed as scheduled on GD 20. A transient mean group food consumption decrease was also observed at the middle dose (70 mg/kg bw/day) for only the first 2 days on test (GD 6–7) during which food consumption was 44% of control.

The 700 mg/kg bw/day group displayed significant 8, 22, and 30% reductions of mean body weight on GD 14, 18, and 20, respectively, compared with the control group. In the 70 mg/kg bw/day group, mean body weight gain was significantly reduced to 44% of control at GD 6 to 10. At 10 mg/kg bw/day, body weights were not significantly altered.

Clinical signs of toxicity were observed in the 700 mg/kg bw/day dose group and included salivation (13/27), oral/nasal discharge (12/27), ptosis (11/27), swollen abdomen (8/27), and blood on the vulva (7/27). These findings were not observed in the other dose groups. At necropsy, the 700 mg/kg bw/day dams also presented with visceral abnormalities including enlarged stomachs (26/27), enlarged adrenals (12/27), and discolored lungs (3/27). No treatment-related visceral abnormalities were noted at 10 or 70 mg/kg bw/day.

In surviving dams treated with 700 mg/kg bw/day, fetal body weight was decreased by 44% in males and 45% in females (Table 2). There were no increases in fetal malformations or variations in any dose group; however, skeletal evaluation was not performed in the high-dose (700 mg/kg bw/day) fetuses due to the extremely reduced fetal weights and delayed ossification in this dose group (Table 3).

In the 1989 rat developmental toxicity study of ATR, one high-dose (100 mg/kg/day) dam was found dead on GD 20 before scheduled necropsy. There was no evidence to suggest this death was treatment related, because the animal displayed neither clinical signs nor abnormal necropsy observations. All other dams survived until study termination. Maternal food consumption was significantly diminished by 13% in the 100 mg/kg bw/day dose group on GD 6 to 15 (Table 4). Food consumption was not altered at ATR dose levels of 5 or 25 mg/kg bw/day. Maternal body weights (corrected for uterus and fetuses) and weight gains were significantly diminished

Table 3
1984 Rat Developmental Toxicity Study of Atrazine: Malformations and Variations[a]

End point	Atrazine dose (mg/kg bw/day)			
	0	10	70	700
Number of fetuses/litters used for external examination	292/23	314/23	349/25	21/5[b]
Gross malformations	0/0	0/0	0/0	0/0
Number of fetuses/litters used for visceral examination	89/22	97/22	105/25	21/5
Diaphragmatic hernia	0	1/1	0	0
Possible hydronephrosis	1/1	0	0	0
Visceral variations[c]	22/14	35/22	35/25	0
Number of fetuses/litter used for skeletal examination	203/23	217/23	244/25	NE[d]
T-13 rudimentary rib	6/5	1/1	2/2	
Polydactyly	0	1/1	0	
Centrum/vertebra agenesis	3/3	0	0	
Rib agenesis	0	1/1	0	
Skeletal variations[e]	203/23	217/23	244/25	

[a]Published as Infurna et al. (1988).
[b]Only five litters were examined in the high-dose group due to maternal mortality.
[c]Short or absent renal papilla and dilated ureter.
[d]Not examined at the discretion of the Study Director, skeletal examinations were not performed due to the extremely reduced fetal weights and subsequent delayed ossification.
[e]Delayed ossification; vertebral centra or sternebrae bipartite, misaligned, or fused; ribs rudimentary, wavy, bifurcated, or cervical.

Table 4
1989 Rat Developmental Toxicity Study Of Atrazine: Fertility and Developmental End Points

End point	Atrazine dose (mg/kg bw/day)			
	0	5	25	100
Total number of mated females	26	26	26	26
Total number of pregnant females	26	25	25	22
Percent of pregnant females	100	96.2	96.2	84.6
Percent of maternal food consumption (GD 6–15)	100	95.8	96.6	87.2*
Percent of maternal body weight gain (GD 6–20)[a]	100	95.8	92.2	79.8*
Mean no. of corpora lutea ± SD	17.7 ± 2.1	17.7 ± 2.1	16.9 ± 2.0	18.3 ± 2.4
Mean no. of implantation sites ± SD	14.0 ± 2.5	14.6 ± 2.0	14.6 ± 2.2	15.9 ± 2.6*
Number of litters examined	26	25	24	21
Mean no. of early resorptions ± SD	0.5 ± 0.7	0.8 ± 1.0	0.5 ± 0.9	0.6 ± 0.8
Mean no. of total resorptions ± SD	0.6 ± 0.7	0.8 ± 1.0	0.5 ± 0.9	0.7 ± 0.9
Mean no. of dead fetuses per litter	0	0	0	0
Mean no. of live fetuses per litter ± SD	13.4 ± 2.5	13.8 ± 2.1	14.5 ± 1.8	15.4 ± 2.9*
Postimplantation loss (%)[b]	4.1	5.5	3.4	4.5
Sex ratio (% males)	50.4	45.2	49.0	57.7
Mean fetal body weight (g) ± SD, males	3.5±0.24	3.6±0.27	3.6±0.27	3.5±0.20
Females	3.3±0.76	3.4±0.25	3.4±0.21	3.3±0.21

[a]Terminal body weight on gestation day 20 minus uterus and conceptus weight.
[b]Preimplantation loss data not calculated in the original manuscript. No treatment-related effect was suggested on review of the tables in the study report.
*Different from the control group at $p \leq 0.05$.

by 20% in animals given 100 mg/kg/day on GD 6 to 20 (Fig. 2; Table 4). No statistically significant effect on body weight or body weight gain was observed in females dosed with ≤25 mg/kg/day. A statistically significant increase in implantations and live fetuses in the 100 mg/kg bw/day dose group was identified and may have been spurious. There were no treatment-related effects on any reproductive parameter or on fetal sex ratio (Fig. 2). There were no significant effects on group mean fetal body weights or fetal development.

The few gross malformations that were observed in this study occurred in the vehicle control group (Table 5). Visceral malformations were noted in the control and 5 mg/kg bw/day dose groups. Fetal visceral variations were confined to the kidneys and ureters and occurred in all groups, including the control group. No skeletal malformations were found in any of the fetuses, and skeletal variations were distributed across all groups, including the control.

Developmental Toxicity Study of ATR in Rabbits

There were three unexpected deaths of treated does, all in the low-dose ATR group (1 mg/kg bw/day). Two deaths appear to have resulted from dosing accidents, and

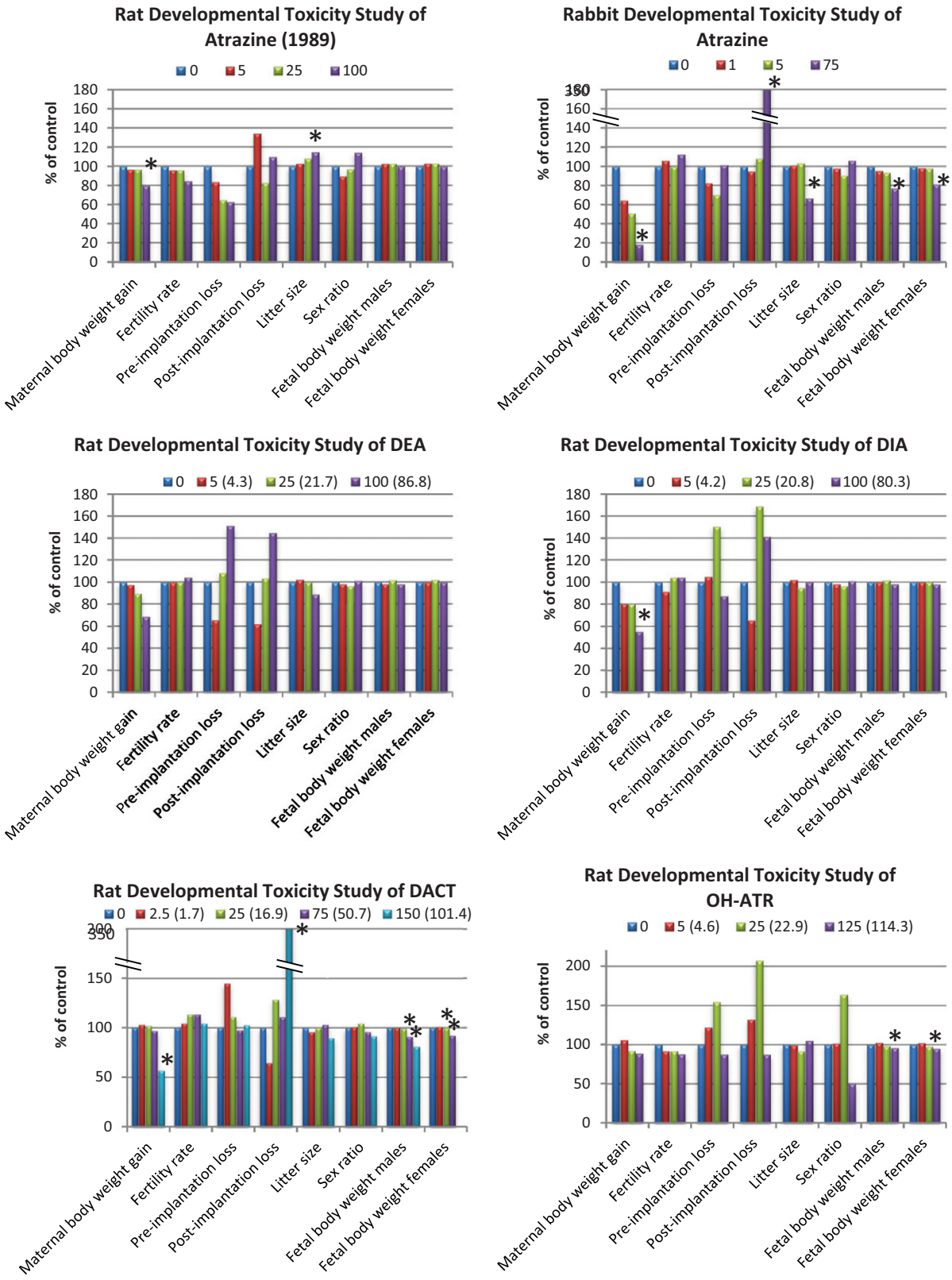

Fig. 2. Selected results of the developmental toxicity studies of atrazine and metabolites in rats and of atrazine in rabbits. Dose levels are expressed in mg/kg bw/day (atrazine equivalent doses in parentheses). Data expressed relative to control values. *$p \leq 0.05$ or lower compared to control. The rabbit developmental toxicity study was published as Infurna et al. (1988).

Table 5
1989 Rat Developmental Toxicity Study of Atrazine: Malformations and Variations

End point	Atrazine dose (mg/kg bw/day)			
	0	5	25	100
Number of fetuses/litters used for external examination	349/26	345/25	347/24	324/21
Anophthalmia, microphthalmia	1/1	0	0	0
Ectrodactyly, filament tail	1/1	0	0	0
External variation (hematoma)	1/1	0	0	0
Number of fetuses/litters used for visceral examination	168/26	167/25	168/24	158/21
Visceral malformation (stomach, liver, kidney, adrenal, spleen irregular shape, reduced size, or agenesis)	2/1	1/1	0	0
Visceral variations (kidney, ureter)	33/14	29/12	22/10	41/13
Number of fetuses/litters used for skeletal examination	181/26	178/25	179/24	166/21
Skeletal variation[a]	181/26	177/25	179/24	166/21

[a]Mainly delayed ossification of skull, ribs, sternebrae, pelvis, forepaw, and hindpaw.

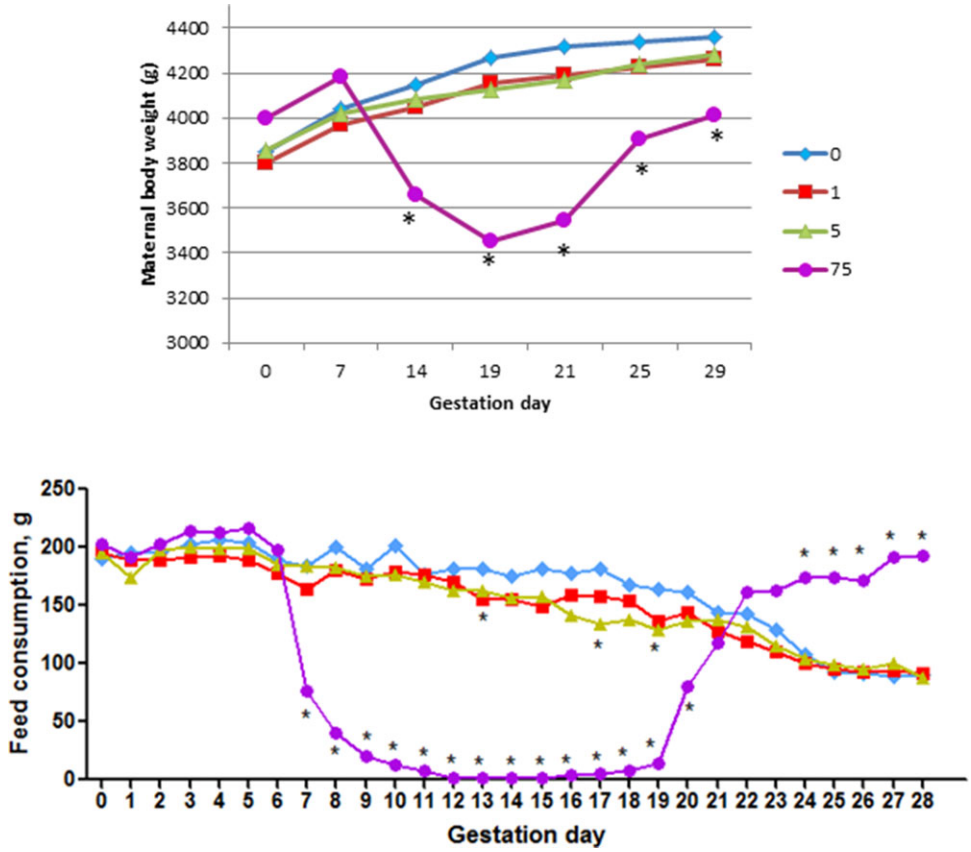

Fig. 3. Maternal body weight (top) and food consumption (bottom) during the rabbit developmental toxicity study of atrazine. Dose groups are expressed in mg/kg bw/day. *$p \leq 0.05$ compared to control. Figures redrawn from data tables in Infurna et al. (1988).

one doe was found dead and was possibly aborting. Three other does were killed late in the study because they were aborting: one in the middle dose group (5 mg/kg/day) and two in the high-dose group (75 mg/kg/day). All other does were killed as scheduled on GD 29. Maternal food consumption was significantly decreased in does administered the 75 mg/kg bw/day dose level of ATR on GD 7 to 20 (Fig. 3); after treatment ended (GD 19), evidence of recovery was observed. A statistically significant reduction of maternal body weight to about 80% of control at its greatest was observed in the 75 mg/kg bw/day dose group from GD 14 through study termination (Fig. 3). Body weight change was negative in the 75 mg/kg bw/day dose group during the treatment period but became positive after the treatment period; however, body weight gain over the pregnancy corrected for uterine weight remained depressed by 320 g compared to control (Figs. 2 and 4). In the middle dose group (5 mg/kg bw/day), mean body weight gains were also significantly diminished from GD 14 to 19 (Fig. 4). In the

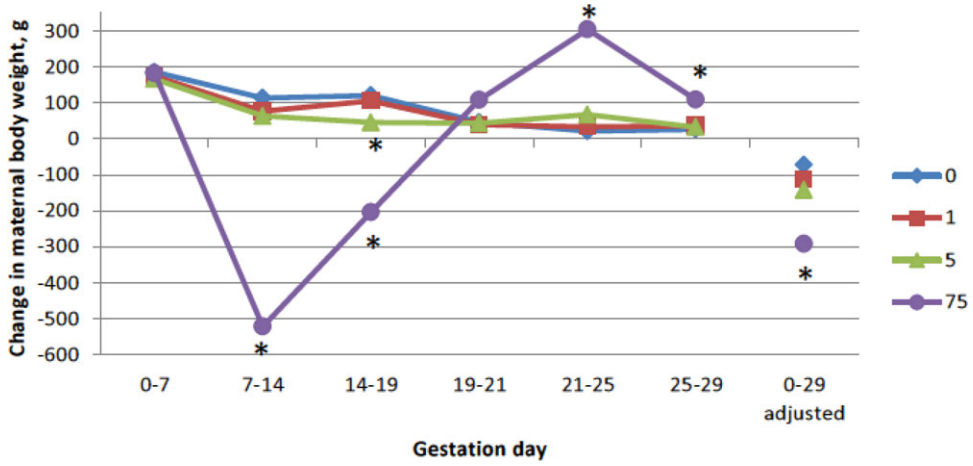

Fig. 4. Change in maternal body weight during the rabbit developmental toxicity study of atrazine. Dose groups are expressed in mg/kg bw/day. Adjusted body weight is corrected for the weight of the pregnant uterus. *$p \leq 0.05$ compared to control. Figure redrawn, data published as Infurna et al. (1988).

Table 6
Rabbit Developmental Toxicity Study of Atrazine: Fertility and Developmental End Points[a]

	Atrazine dose (mg/kg bw/day)			
End point	0	1	5	75
Number of inseminated females	19	19	19	19
Number (%) maternal mortality	0	3 (15.8)	1 (5.3)[b]	2 (10.5)[b]
Number of pregnant females	16	17	16	18
Percent pregnant females	84	89	84	95
Mean no. of corpora lutea/litter ± SD	13.6 ± 1.7	13.1 ± 2.4	12.9 ± 2.4	14.3 ± 3.1
Mean no. of implantations/litter ± SD	10.1 ± 2.4	10.2 ± 2.6	10.5 ± 2.0	10.4 ± 3.5
No. of litters examined	16	14	15	15[c]
Mean no. of resorptions ± SD	1.3 ± 1.2	1.4 ± 1.8	1.4 ± 1.2	4.8 ± 3.4*
Mean no. of dead fetuses ± SD	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Mean no. of preimplantation loss ± SD	3.6 ± 2.2	2.9 ± 2.6	2.5 ± 2.1	3.9 ± 3.7
Percent preimplantation loss	26.1	21.6	18.4	26.5
Percent postimplantation loss	12.0	11.4	13.0	42.6*
Mean no. of live fetuses ± SD	8.8 ± 2.1	8.9 ± 2.2	9.1 ± 1.9	5.9 ± 3.4*
Fetal sex ratio (% males)	48.6	47.6	44.1	51.7
Mean fetal body weight (g) ± SD[d], males	46.1 ± 5.5	44.0 ± 6.1	43.2 ± 6.0	35.7 ± 5.8*
Mean fetal body weight (g) ± SD, females	44.0 ± 3.7	43.3 ± 5.4	43.1 ± 4.5	35.8 ± 6.2*

[a]Published as Infurna et al. (1988).
[b]Killed due to abortion.
[c]One additional litter was totally resorbed.
[d]Calculated from the study report, the published manuscript reports SEM.
*Different from the control at $p \leq 0.05$.

middle and low-dose groups (1 and 5 mg/kg bw/day), no statistically significant reduction of body weight gain was apparent. All does in the 75 mg/kg bw/day dose group exhibited significant stool changes. Stool changes were also observed to some degree in the other dose groups. There was blood on the vulva or in the cage of 4/19 75 mg/kg bw/day dose group does. No significant treatment-associated gross pathology findings were observed at necropsy.

In the 75 mg/kg bw/day dose group only, there was a 3.7-fold increase in the number of fetal resorptions and a 33% decrease in the number of viable fetuses (Table 6). No treatment-related changes in the number of corpora lutea or implantation sites were noted. Statistically significant reductions of mean body weight were observed in male fetuses (67% of control body weight) and female fetuses (81% of control body weight; Table 6). The sex ratio of the fetuses was not significantly different from the expected 1:1 M/F ratio. Of 489 fetuses evaluated in this study, only two were grossly malformed (Table 7). One control fetus had an omphalocele, and one 75 mg/kg bw/day dose group fetus had ablepharia. One visceral malformation (absent gallbladder) was observed in a fetus of the 5 mg/kg bw/day dose group (Table 7). One control fetus had a skeletal malformation (ectromelia; Table 7). There were increases in ossification delay of isolated bones in the 75 mg/kg bw/day dose group when evaluated on a litter basis, without a change in the frequency of overall skeletal variations.

Table 7
Rabbit Developmental Toxicity Study of Atrazine: Malformations and Skeletal Variations[a]

End point	Atrazine dose (mg/kg bw/day) 0	1	5	75
Total number of fetuses/ litters examined	140/16	124/14	136/15	89/15
External malformations:	1/1	0	0	1/1
Omphalocele	1/1	0	0	0
Ablepharia	0	0	0	1/1
Visceral malformations:	0	0	1/1	0
Gallbladder absent	0	0	1/1	0
Skeletal findings:				
Ectromelia	1/1	0	0	0
Skeletal variations[b]	94/16	80/13	99/15	75/14*

[a]Published as Infurna et al. (1988). Variations mentioned only for skeletal examination.
[b]Delayed ossification, vertebral centra; sternebrae additional, bipartite, misaligned, or fused; ribs rudimentary, wavy, bifurcated, or additional.
*Delayed ossification of patella and some paw bones statistically different from control at $p \leq 0.05$.

Developmental Toxicity Study of DIA in Rats

All animals survived to necropsy, except for one control animal found dead on GD 13. Maternal body weight gain of animals given 100 mg/kg/day (high-dose group) was significantly decreased to 55.4% of control from GD 6 through 21 (Table 8). Group mean daily food consumption was significantly reduced in the 100 mg/kg bw/day (by 20.4%) and 25 mg/kg bw/day (by 6.1%) dose groups during treatment (Table 8).

Maternal necropsy at GD 21 revealed no remarkable pathologic findings. Survival, pregnancy status, and findings at cesarean section are presented in Table 8. There was no significantly increased fetal loss related to treatment. The numbers of corpora lutea and implantation sites were comparable for all groups. The numbers of live fetuses, sex ratio, and group mean fetal body weights were comparable in all groups (Fig. 2; Table 8). One fetus in the 25 mg/kg bw/day group had an omphalocoele; no other external malformations were seen (Table 9). There were single-fetus observations of dilated nasal cavity in the 25 and 100 mg/kg bw/day dose groups and renal pelvis dilatation was identified in the control and 100 mg/kg bw/day dose group. No skeletal malformations were observed in this study. An increase in ossification delay was noted in isolated bones in the 100 mg/kg bw/day dose group, but there was no pattern of affected bones that might indicate a cause for concern.

Developmental Toxicity Study of DEA in Rats

All animals survived to necropsy except for one dam in the middle (25 mg/kg/day) dose group found dead on GD 10; at necropsy, no pathological signs were found. Percent maternal weight gains were significantly reduced compared to control during part of the treatment period in the 25 and 100 mg/kg bw/day dose groups. In the 25 mg/kg bw/day dose group, there was a statistically significant 17% decrease in maternal weight gain on GD 6 to 11. In the 100 mg/kg bw/day dose group, there was a 59% decrement in maternal weight gain on GD 6 to 11 and a 13% decrease in maternal body weight gain on GD 11 to 16. Food consumption was decreased by 9% in the 25 mg/kg bw/day dose group and by 30% in the 100 mg/kg bw/day dose group on GD 6 to 11. There were no statistically significant changes in food consumption over the entire treatment period or in maternal body weight gain corrected for uterine weight (Table 10). Maternal necropsy at GD 21 revealed no remarkable

Table 8
Rat Developmental Toxicity Study of Deisopropylatrazine (DIA): Fertility and Developmental End Points

End point	DIA dose in mg/kg bw/day (atrazine equimolar dose)[a] 0	5 (4.2)[a]	25 (20.8)	100 (80.3)
Number of mated females	24	24	24	24
Number of pregnant females	23	21	24	24
Percent of pregnant females	95.8	87.5	100	100
Percent of maternal food consumption (GD 6–15)	100	95.9	93.9*	79.6*
Percent of maternal body weight gain (GD 6–21)[b]	100	80.5	80.2	55.4*
Mean no. of corpora lutea ± SD	16.4 ± 2.3	16.6 ± 1.4	17.0 ± 1.7	16.4 ± 2.3
Mean no. of implantation sites ± SD	14.8 ± 2.0	14.9 ± 1.8	14.5 ± 3.3	15.0 ± 2.3
Number of litters examined	22	21	23	23
Mean no. of early resorptions ± SD	0.8 ± 0.9	0.6 ± 1.0	1.2 ± 3.0	1.0 ± 2.2
Mean no. of late resorptions ± SD	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.2
Mean no. of total resorptions ± SD	0.8 ± 0.9	0.6 ± 1.0	1.2 ± 3.2	1.0 ± 2.2
Mean no. of dead fetuses per litter	0	0	0	0
Mean no. of live fetuses ± SD	14.0 ± 2.4	14.3 ± 2.0	13.3 ± 4.5	14.0 ± 3.8
Postimplantation loss (%)	5.8	3.8	9.8	8.2
Sex ratio (% males)	50.6	49.7	48.8	51.2
Mean fetal body weight (g) ± SD, males	5.6 ± 0.3	5.6 ± 0.3	5.7 ± 0.4	5.5 ± 0.3
Females	5.3 ± 0.3	5.3 ± 0.3	5.3 ± 0.4	5.2 ± 0.3

[a]Atrazine equimolar dose in parenthesis; DIA has a molecular weight of 173.6 g.
[b]Terminal body weight on gestation day 21 minus uterus and conceptus weight.
*Different from the control group at $p \geq 0.05$.

Table 9
Rat Developmental Toxicity Study of Deisopropylatrazine (DIA): Malformations and Variations

	DIA dose in mg/kg bw/day (atrazine equimolar dose)[a]			
End point	0	5 (4.0)	25 (20.1)	100 (80.5)
Total no. of fetuses/litters examined for external examination	308/22	300/21	320/23	336/23
Total no. of fetuses with external malformations	0	0	1/1	0
Omphalocele	0	0	1/1	0
External variations	0	0	0	0
No. of fetuses/litters used for visceral examination	148/22	145/21	155/23	164/23
Total no. of fetuses/litters with malformations	4/2	0	1/1	3/3
Nasal cavities dilated	0	0	1/1	1/1
Renal pelvic dilatation	4/2	0	0	2/2
Visceral variations	0	0	0	0
No. of fetuses/litters subjected to skeletal examination	160/22	155/21	165/22	172/23
Total no. of fetuses with malformations	0	0	0	0
Total no. of fetuses/litters with skeletal variations[b]	160/22	155/21	164/22[c]	172/23[c,d]

[a]Atrazine equimolar dose in parenthesis; DIA has a molecular weight of 173.6 g.
[b]Mostly delayed ossification.
[c]Fused sternebrae 1 and 2 and shortened rib 13 increased compared to control $p \leq 0.05$.
[d]Delayed ossification of sternebra 2 and phalanges of some hindpaw digits increased compared to control at $p \leq 0.05$.

Table 10
Rat Developmental Toxicity Study of Desethylatrazine (DEA): Fertility and Development

	DEA dose in mg/kg bw/day (atrazine equimolar dose)[a]			
End point	Control	5 (4.3)	25 (21.7)	100 (86.8)
Number of mated females	24	24	24	24
Number of pregnant females	23	23	23	24
Percent pregnant females	95.8	95.8	95.8	100
Percent of maternal food consumption (GD 6–15)	100	96.5	94.8	80.3
Percent of maternal body weight gain (GD 6–21)[b]	100	86.0	73.3	79.5
Mean no. of corpora lutea ± SD	15.7 ± 1.7	15.9 ± 1.7	16.1 ± 1.8	15.0 ± 2.5
Mean no. of implantation sites ± SD	14.8 ± 2.0	15.3 ± 1.4	15.1 ± 1.8	13.7 ± 2.5
Number of litters examined	23	23	22	24
Mean no. of live fetuses ± SD	14.2 ± 2.0	14.5 ± 1.6	14.2 ± 2.3	12.6 ± 3.1
Mean no. of early resorption ± SD	0.6 ± 0.8	0.8 ± 0.9	0.9 ± 1.1	1.0 ± 1.3
Mean no. of late resorption ± SD	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3
Mean no. of total resorption ± SD	0.6 ± 0.8	0.8 ± 0.9	0.9 ± 1.1	1.1 ± 1.3
Mean no. of dead fetuses ± SD	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Percent of preimplantation loss ± SD	5.8 ± 7.1	3.6 ± 4.2	6.0 ± 6.4	8.4 ± 5.6
Percent of postimplantation loss ± SD	4.0 ± 5.4	5.1 ± 5.7	6.3 ± 8.2	8.8 ± 11.7
Sex ratio (% males)	50.8	49.7	48.7	50.5
Mean fetal body weight (g) ± SD, males	5.7 ± 0.3	5.6 ± 0.3	5.8 ± 0.3	5.6 ± 0.3
Females	5.3 ± 0.3	5.3 ± 0.3	5.4 ± 0.3	5.3 ± 0.3

[a]Atrazine equimolar dose in parenthesis; DEA has a molecular weight of 187.6 g.
[b]Body weight change on gestation day 21 minus uterus and conceptus weight.

pathologic findings. Survival, pregnancy status, and findings at cesarean section were unaffected by treatment (Table 10). There was no fetal loss related to treatment. The numbers of corpora lutea and implantation sites were comparable for all treated groups as were pre- and postimplantation loss, sex ratio, and fetal body weight (Fig. 2).

Fetal observations are summarized in Table 11. Two fetuses in the middle dose group (25 mg/kg bw/day) had omphalocoeles; one of these fetuses also had hindlimb agenesis. One low-dose (5 mg/kg bw/day) fetus had a kinked tail. No other external malformations were seen. Dilatation of the renal pelvis was seen in one fetus of the 25 mg/kg bw/day dose group, and bilateral hydronephrosis was observed in one fetus of the 100 mg/kg bw/day dose group (Table 11). No relevant skeletal findings were observed except in the fetus with hindlimb agenesis. An increase in ossification delay in the 100 mg/kg bw/day dose group was confined to the proximal phalanx of posterior digit 3. Fusion of sternebrae 1 and 2 and shortened 13th rib were also noted in this dose group.

Developmental Toxicity Study of DACT in Rats

There were no unscheduled deaths, treatment-related clinical signs, or necropsy observations in this study. A significant 26.9% reduction in maternal food

Table 11
Rat Developmental Toxicity Study of Desethylatrazine (DEA): Malformations and Variations

	DEA dose in mg/kg bw/day (atrazine equimolar dose)[a]			
End point	0	5 (4.3)	25 (21.7)	100 (86.8)
Total no. of fetuses/litters used for external examination	327/23	334/23	313/22	303/24
No. of fetuses/litters with any external malformation	0	1/1	3/2	0
Hindlimb agenesis	0	0	1/1	0
Kinked tail	0	1/1	0	0
Omphalocele	0	0	2/2	0
External variations	0	0	0	0
Total no. of fetuses/litters used for visceral examination	159/23	160/23	149/22	145/24
No. of fetuses/litters with any visceral alteration	0	0	1/1	1/1
Bilateral hydronephrosis	0	0	0	1/1
Visceral variation (renal pelvic dilatation)	0	0	1/1	0
Total no. of fetuses/litters used for skeletal examination	168/23	174/23	164/22	158/24
No. of fetuses/litters with any skeletal malformation	0	0	1/1[b]	0
Pelvic girdle (missing ischium)	0	0	1/1[b]	0
Pelvic girdle (missing pubis)	0	0	1/1[b]	0
Missing tibia	0	0	1/1[b]	0
Missing fibula	0	0	1/1[b]	0
Missing hind paw	0	0	1/1[b]	0
No. of fetuses/litters with skeletal variations[c]	168/23	173/23	163/22	158/24[d]

[a]Atrazine equimolar dose in parenthesis; DEA has a molecular weight of 187.6 g.
[b]Same fetus had all these malformations and omphalocele.
[c]Mostly delayed ossification and assymetries.
[d]Fused sternebrae 1 and 2, shortened rib 13, delayed ossification of one phalanx increased compared to controls at $p \leq 0.05$.

Table 12
Rat Developmental Toxicity Study of Diaminochlorotriazine (DACT): Fertility and Development

	DACT dose in mg/kg bw/day (atrazine equimolar dose)[a]				
End point	Control	2.5 (1.7)	25 (16.9)	75 (50.7)	150 (101.4)
Number of mated females	26	26	26	26	26
Number of pregnant females	22	23	25	25	23
Percent pregnant females	84.6	88.5	96.2	96.2	88.5
Percent of maternal food consumption (GD 6–15)	100	105	101	97.6	73.1*
Percent of maternal body weight gain (GD 6–20)[b]	100	106	110	89.8	56.7*
Mean no. of corpora lutea ± SD	16.0 ± 3.1	16.0 ± 2.4	16.6 ± 1.7	16.4 ± 1.9	17.2 ± 4.4
Mean no. of implantation sites ± SD	14.0 ± 1.6	13.1 ± 3.3	14.2 ± 1.8	14.4 ± 1.9	14.0 ± 3.3
Number litters examined	22	23	25	25	23
Mean no. of live fetuses ± SD	13.2 ± 1.7	12.6 ± 3.3	13.2 ± 1.9	13.6 ± 2.3	11.3 ± 4.2
Mean no. of early resorption ± SD	0.8 ± 0.7	0.5 ± 0.9	1.0 ± 1.2	0.8 ± 1.1	2.2 ± 3.4
Mean no. of late resorption ± SD	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 1.3
Mean no. of total resorption ± SD	0.8 ± 0.7	0.5 ± 0.9	1.0 ± 1.2	0.8 ± 1.1	2.6 ± 3.7*
Mean no. of dead fetuses ± SD	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.4
Mean postimplantation loss ± SD	0.8 ± 0.7	0.5 ± 0.9	1.0 ± 1.2	0.8 ± 1.1	2.7 ± 3.7
Percent postimplantation loss	5.6	3.6	7.2	6.2	18.9*
Sex ratio (% males)	50.7	51.0	53.0	48.5	46.7
Mean fetal body weight (g) ± SD, males	3.45 ± 0.06	3.45 ± 0.06	3.43 ± 0.06	3.14 ± 0.06*	2.79 ± 0.06*
Females	3.29 ± 0.05	3.32 ± 0.05	3.29 ± 0.05	3.03 ± 0.05*	2.68 ± 0.05*

[a]Atrazine equimolar dose in parenthesis; diaminochlorotriazine has a molecular weight of 145.6 g.
[b]Terminal body weight on gestation day 20 minus uterus and conceptus weight.
*Different from the control group at $p \leq 0.05$.

consumption was observed in the high-dose group (150 mg/kg bw/day) during GD 6 to 15 (Table 12). In the 75 mg/kg bw/day dose group, feed consumption was reduced by 19% during one interval (GD 6–8). Following the completion of dosing, food consumption among treated groups returned to control group levels.

Reduced food consumption was correlated with reduced maternal body weight. In the 150 mg/kg bw/day group, mean maternal body weights were significantly reduced by 8% on GD 8, by 13% on GD 12, by 12% on GD 16, and by 12% on GD 20, and body weight gain in this group, corrected for uterine weight, was reduced by 43% (Table 12). At 75 mg/kg bw/day, body weight gain was 97% of the control body weight gain. There were no compound-related effects on any of the reproductive end points examined at ≤75 mg/kg DACT (Table 12).

Table 13
Rat Developmental Toxicity Study of Diaminochlorotriazine (DACT): Malformations and Variations

End point	DACT dose in mg/kg bw/day (atrazine equimolar dose)[a]				
	0	2.5 (1.7)	25 (16.9)	75 (50.7)	150 (101.4)
Number of fetuses/litters used for external examination	290/22	290/23	330/25	340/25	259/23
Total no. of fetuses/litters with external malformations	0	2/2	0	1/1	2/2
Acaudate	0	0	0	0	1/1
Filamentous tail	0	0	0	1/1	0
Protruding tongue	0	1/1	0	0	0
Umbilical hernia	0	1/1	0	0	1/1
Number of fetuses/litters used for visceral examination	141/22	140/23	160/25	166/25	126/23
Total no. of fetuses/litters with visceral malformations	0	0	1/1	0	0
Interventricular septal defect	0	0	1/1[b]	0	0
Situs inversus (heart)	0	0	1/1[b]	0	0
Unilobular lungs	0	0	1/1[b]	0	0
Total no. of fetuses/litters with visceral variations[c]	44/15	41/17	62/21	50/19	59/19
Number of fetuses/litters used for skeletal examination	149/22	150/23	170/25	174/25	133/23
Total no. of fetuses with malformations	0	0	0	0	0
Total no. of fetuses/litters with skeletal variations[d]	148/22	150/22	170/25	174/25*	133/23*

[a]Atrazine equimolar dose in parenthesis; DACT has a molecular weight of 145.6 g.
[b]Same fetus had all these malformations.
[c]Mostly short or absent renal papillae, dilated uteters.
[d]Mostly ossification delay.
*Almost 100% of fetuses in all dose groups, including the control, demonstrated incomplete ossification of proximal phalanges of the forepaw. Variations were statistically increased ($p \leq 0.05$) over control in the mid- and high-dose groups when forepaw/metacarpal observations were excluded. These variations consisted largely of ossification delays in skull and vertebrae.

However, at 150 mg/kg bw/day, a significant 3.3-fold increase was observed in the number of fetal resorptions and consequently a similar fold increase in postimplantation loss. Group mean fetal weights were significantly reduced at both 75 (by 9%) and 150 (by 19%) mg/kg bw/day (Table 12; Fig. 2).

Table 13 presents a summary of fetal malformations. Of more than 1500 fetuses examined, only five showed any external malformation and none of these was dose related. Only one of 733 fetuses examined had a visceral malformation; this fetus had multiple malformations. No skeletal malformations were observed in any dose group. There was an increase in ossification delay in the two highest dose groups but only when forepaw/metacarpal observations were excluded. These nonlimb malformations primarily involved skull bones and vertebrae.

Developmental Toxicity Study of OH-ATR in Rats

There were no treatment-related deaths or clinical observations in this study nor were there apparent treatment-related necropsy observations. A statistically significant 8.4% reduction in group mean food consumption occurred at the high dose (125 mg/kg bw/day) during GD 6 to 15 (Table 14). There were no treatment-related changes in food consumption in the 25 or 5 mg/kg bw/day dose groups. Over the entire span of gestation, there were no statistically significant alterations in maternal body weight gain in any group; although there was a difference across dose groups in maternal weight gain corrected for uterine weight by analysis of variance (Table 14). There were no significant compound-related effects on any of the reproductive parameters examined in this study. Fetal body weights were significantly reduced in the 125 mg/kg bw/day dose group by less than 0.2 g, or about 4 to 5% (Table 14; Fig. 2). Sex ratios were unaffected. Two of 311 fetuses from two different high-dose (125 mg/kg bw/day) litters showed external malformations consisting of gastroschisis in one and umbilical hernia in the other (Table 15). With the exception of a middle dose (25 mg/kg bw/day) fetus with cleft palate, none of the treated or control fetuses displayed any visceral or skeletal malformations. There was an increase in delayed ossification of specific bones in the 125 mg/kg bw/day dose group. There was no pattern of affected bones that might indicate a cause for concern.

DISCUSSION

This article presents data from seven embryo-fetal toxicity studies performed with ATR or its metabolites. These studies showed no statistically significant increase in developmental abnormalities even at dose levels associated with maternal toxicity. To put these studies in context, human drinking water exposure to ATR from ingesting 2 l/day of drinking water with the maximum permissible concentration of 3 μg/l (U.S. Environmental Protection Agency, 2012) would be 6 μg/day or 0.1 μg/kg bw/day for a 60-kg woman, which is 1000 times lower than the rat developmental no effect level and 50 times less than that of the rabbit (based on increased resorptions and decreased fetal weights). The World Health Organization Drinking Water Standard for ATR and its chlorinated metabolites is 0.1 μg/ml (100 ppb; WHO, 2011).

The 1984 rat developmental toxicity study used a high ATR dose level of 700 mg/kg bw/day, which resulted in the death of 21/27 dams before scheduled termination. This dose level was well above the MTD. None of the other dose levels reached the MTD. Because of the poor growth of fetuses in the 700 mg/kg bw/day dose

Table 14
Rat Developmental Toxicity Study of Hydroxyatrazine (OH-ATR): Fertility and Development

	Hydroxyatrazine dose in mg/kg bw/day (atrazine equimolar dose)[a]			
End point	0	5 (4.6)	25 (22.9)	125 (114.3)
Number of mated females	26	26	26	26
Number of pregnant females	25	23	23	22
Percent pregnant females	96.2	88.5	88.5	84.6
Percent of maternal food consumption (GD 6–15)	100	103	97.3	91.6*
Percent of maternal body weight gain (GD 6–20)[b]	100	111	94.5	86.5
Mean no. of corpora lutea ± SD	16.4 ± 3.3	17.7 ± 2.5	17.4 ± 2.8	17.2 ± 2.4
Mean no. of implantation sites ± SD	13.9 ± 3.2	14.4 ± 2.9	13.3 ± 4.3	14.9 ± 1.5
Number of litters examined	25	23	22	22
Mean no. of early resorptions ± SD	0.5 ± 0.7	1.1 ± 1.8	1.0 ± 1.4	0.7 ± 0.8
Mean no. of late resorptions ± SD	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean no. of total resorptions ± SD	0.5 ± 0.7	1.1 ± 1.8	1.0 ± 1.4	0.7 ± 0.8
Mean no. of live fetuses ± SD	13.4 ± 3.4	13.3 ± 3.3	12.3 ± 4.8	14.1 ± 1.7
Mean no. of dead fetuses ± SD	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Postimplantation loss (%)	5.6 ± 10.6	7.4 ± 12.7	11.6 ± 22.6	4.9 ± 5.6
Sex ratio (% males)	49.6	50.7	51.9	50.2
Mean fetal body weight (g) ± SD, males	3.61 ± 0.04	3.71 ± 0.04	3.55 ± 0.05	3.47 ± 0.04*
Females	3.43 ± 0.05	3.51 ± 0.05	3.35 ± 0.05	3.26 ± 0.05*

[a]Atrazine equimolar dose in parenthesis; hydroxyatrazine has a molecular weight of 197.2 g.
[b]Terminal body weight of gestation day 20 minus uterus and conceptus weight, statistically significant difference across dose groups by analysis of variance at $p \leq 0.05$; statistical significance lost after removal of the high-dose group.
*Different from the control group at $p \leq 0.05$.

Table 15
Rat Developmental Toxicity Study of Hydroxyatrazine: Malformations and Variations

	Hydroxyatrazine dose in mg/kg bw/day (atrazine equimolar dose)[a]			
End point	0	5 (4.6)	25 (22.9)	100 (114.3)
Number of fetuses/litters used for external examination	335/25	306/23	283/22	311/22
Total no. of fetuses/litters with external malformations	0	0	0	2/2
Gastroschisis	0	0	0	1/1
Umbilical hernia	0	0	0	1/1
Number of fetuses/litters used for visceral examination	158/24	147/23	136/21	151/22
Total no. of fetuses with visceral malformations	0	0	0	0
Total no. of fetuses/litters with visceral variations[b]	29	31	15	11
Number of fetuses/litters used for skeletal examination	177/25	159/23	147/22	160/22
Total no. of fetuses/litters with skeletal malformations	0	0	1/1	0
Cleft palate	0	0	1/1	0
Total no. of fetuses/litters with skeletal variations	174/24	158/23	147/22	159/22*

[a]Atrazine equimolar dose in parenthesis; hydroxyatrazine has a molecular weight of 197.2 g.
[b]Mostly short or absent renal papillae, dilated uteters.
*Decreased ossification with respect to control of some phalanges and metacarpals, interparietal, and hyoid, $p \leq 0.05$.

group, skeletal examinations were not performed in this dose group. There was no evidence of a dose-related increase in malformations, even among the surviving fetuses of the 700 mg/kg bw/day dose group although maternal mortality in this group permitted the examination of only a few fetuses. A second rat developmental toxicity study in 1989 used dose levels that were more appropriate with about a 20% decrease in corrected maternal body weight gain (exclusive of uterine weight) in the 100 mg/kg bw/day high-dose group. There was no treatment-associated increase in adverse developmental effects in this study.

In the rabbit developmental toxicity study, the high-dose level of 75 mg/kg bw/day produced a statistically significant decrease in maternal body weight during the treatment period that did not return to control levels after treatment (Figs. 3 and 4). This reduction in maternal body weight was likely due to lower food consumption, which fell to nearly zero between GD 10 and 19. There were no statistically significant effects of treatment at lower dose levels on maternal body weight. The rate of fetal resorption and postimplantation loss was increased in the 75 mg/kg bw/day dose group. Fewer live fetuses were born, and the survivors weighed less than the controls. The lower dose levels (1 and 5 mg/kg bw/day) were without significant effect. There was no change in congenital malformations even at the maternally toxic and embryotoxic dose level of 75 mg/kg bw/day.

ATR is metabolized in animals by removal of one of the two alkyl side groups to DIA or DEA. Removal of both groups produces DACT. Plants often metabolize ATR in a different way, by substituting the chlorine atom with a hydroxyl group to make OH-ATR, which is no longer an active herbicide. Developmental toxicity studies of all four ATR metabolites were conducted using pregnant rats. Each mammalian metabolite was administered at a maximum of 100 mg/kg bw/day except for DACT, the highest dose of which was 150 mg/kg bw/day. In the case of all three mammalian metabolites, the highest administered dose exceeded the MTD, that is, each produced a greater than 10% reduction of dam body weight gain compared to the respective control groups.

The highest dose level (120 mg/kg bw/day) of the plant metabolite OH-ATR, which was equivalent on a molar basis to the highest tested dose levels of DIA and DEA, did not significantly suppress dam body weight gain by the planned statistical analytical methods; however, analysis of variance across dose groups gave a p value of 0.04. Statistical significance in the analysis of variance was lost when the 125 mg/kg bw/day dose group was removed, suggesting that the apparent 13.5% decrement in maternal weight gain in this dose group may have been real. Inasmuch as maternal food consumption was reduced during the treatment interval and mean fetal body weight was reduced at this dose level, this dose level was appropriate for developmental toxicity testing. Moreover, the possible 13.5% decrease in maternal weight gain in this dose group (depending on statistical method) may be sufficient to explain the 4 to 5% decrease in fetal weight.

None of the metabolites exerted any apparent effect on reproductive performance, even in the presence of maternal toxicity. Average fetal weights were significantly lowered by DACT at 75 and 150 mg/kg bw/day (Table 12) and by OH-ATR at 125 mg/kg bw/day (Table 14) but not by the mono-dealkylated metabolites. None of the metabolites increased fetal malformations even at dose levels exceeding the MTD.

Skeletal variations in these studies consisted predominantly of delays in ossification, statistically significant for isolated bones at the highest dose levels. Ossification delay is typically seen in high-dose groups in the presence of maternal or fetal toxicity and does not have teratological significance, because it does not interfere with viability or function and because it is reversible (Carney and Kimmel, 2007).

Current test guidelines include exposure to the test compound until the end of gestation. The protocols used in the studies presented here administered ATR until the end of hard palate closure, an earlier design. These designs may be insensitive to possible late pregnancy effects of ATR, including possible effects on reproductive organ development. The ATR multigeneration studies have addressed such late pregnancy effects (DeSesso et al., 2014).

A comprehensive review of published literature on the developmental effects of ATR on amphibians, reptiles, and fish has just been completed (Van der Kraak et al., 2014, submitted). We will focus on a limited subset of the studies with developmental end points for pregnant rats, mouse preimplantation embryos, avian eggs, fish, and frogs.

A study in an unspecified strain of rat used subcutaneous administration of ATR on GD 3, 6, and 9 at dose levels up to 2000 mg/kg bw/day (Peters and Cook, 1973). Dams were permitted to litter. There was a reduction in the number of pups per litter at 800 and 2000 mg/kg bw/day. Little experimental detail was given in this report, and group sizes were only three or four dams per dose group. The experiments reported in the current article indicate that the dose levels used by Peters and Cook may have been above the MTD.

Culture of mouse embryos for 96 hr beginning at the one-cell pronuclear stage in medium containing ATR 0.035 μg/ml (35 ppb) increased the percent cells showing apoptosis without decreasing the number of cells per embryo (Greenlee et al., 2004). A decrease in the proportion of embryos reaching the blastocyst stage was shown in one of two replicates. The findings of this study cannot be compared to the developmental studies discussed in the current article, because in the whole animal studies, there was no ATR treatment during the preimplantation period. The findings from the in vitro preimplantation embryo study are not supported by the results of the multigeneration studies (DeSesso et al., 2014). The concentration of ATR used in the 96-hr cultures was 11.7 times the maximum permissible ATR drinking water concentration in the United States. It is unlikely that the concentration that was used in vitro could be achieved in oviductal and uterine fluids in women exposed to ATR in drinking water, although we are not aware of data on oviductal or uterine fluid ATR concentrations.

Zebrafish embryos have been proposed as a model for screening or mechanistic studies in developmental toxicology. The effective concentrations of ATR for interference with zebrafish development have been reviewed (McCollum et al., 2011). The median lethal concentration for the embryo is 1255 μM (27 mg/ml). A decrease in body size occurs at a lowest effective concentration of 200 μM (4.3 mg/ml) from 6 to 48 hr postfertilization. The lowest concentration at which morphological abnormalities occurs is 20 μM (0.43 mg/ml; 430 ppm). This concentration is more than 5 orders of magnitude higher than the permissible ATR drinking water concentration in the United States. Neither the review nor the underlying studies indicated a No Observed Effects Concentration (NOEC; Wiegand et al., 2001; Ton et al., 2006; McCollum et al., 2011).

In the Frog Embryo Teratogenesis Assay—Xenopus, the median lethal concentration of ATR is 0.2 to 31.8 mg/l (0.9–147 μM), and the median teratogenic concentration of ATR is 0.1 to 9.3 (0.4–43 μM), with variation depending on the *Xenopus* species, incubation temperature, and replicate (Fort et al., 2004). The ratio between the median lethal and teratogenic concentration is 2.0 to 9.3. These results might be used to prioritize ATR for in vivo mammalian testing. Inasmuch as such testing has been completed, the Frog Embryo Teratogenesis Assay—Xenopus results do not add to human health risk assessment and are not considered appropriate for use in a regulatory setting (Spielmann, 2005).

Immersion of day 3 mallard eggs for 30 sec in an ATR emulsion did not produce lethality or malformations at the highest emulsifiable concentration (Hoffman and

Albers, 1984). It is difficult to compare these findings to the mammalian developmental tests due to differences in exposure routes, absence of influence of both the maternal organism and the placenta, and imprecision of the ATR dose delivered by immersion.

Epidemiological studies have raised questions regarding the association of ATR exposure and various birth defects, fetal growth restriction, and miscarriage (reviewed by Goodman et al., 2014). The experimental animal studies presented here were used to assess the biological plausibility of ATR as a cause of the adverse effects described in some of the epidemiology studies. An association of birth defects with ATR exposure is not supported by the rat and rabbit studies presented here, which showed no increase in malformations with ATR treatment in two species at or above the MTD and no increase in malformations with DIA, DEA, DACT, or OH-ATR treatment at or above the MTD.

Gastroschisis, the subject of three epidemiology studies (Goodman et al., 2014), was identified in only one high-dose (120 mg/kg bw/day) fetus in the OH-ATR study out of 6074 fetuses (467 litters) exposed to either parent ATR or one of the four metabolites. Among controls, there were no instances of gastroschisis among 2041 fetuses from 157 litters in these studies. The historical control fetal incidence of gastroschisis in rats is 0.01% with a litter incidence of 0.11% (Lang, 1993).

Omphalocele was described in one control fetus in the rabbit study on ATR, one middle dose (25 mg/kg bw/day) fetus in the DIA study, and two middle dose (25 mg/kg bw/day) fetuses from two litters in the DEA study. Umbilical hernia, which is similar to omphalocele, occurred in one low-dose (2.5 mg/kg bw/day) fetus in the DACT study and one high-dose (120 mg/kg bw/day) fetus in the OH-ATR study. These single-fetus observations of omphalocele and umbilical hernia were not dose related and are unrelated to gastroschisis, which occurs via a different embryological process (Sadler, 2010). Omphalocele occurred in historical control data included with the study reports for the DIA and DEA developmental toxicity studies at a mean fetal incidence of 0.1% (range 0–0.7%) and a mean litter incidence of 0.9% (range 0–4.5%).

A possible association of ATR exposure with human fetal weight reduction is not consistent with rat studies involving much higher levels of ATR exposure. Fetal weight was reduced in rabbits after maternal dose levels of 75 mg/kg/day, at which there were also impairments of maternal weight. The multigeneration ATR studies in rats do not support either male-mediated early pregnancy loss or female-mediated preterm birth with exposure levels up to 500 ppm in the diet, estimated to correspond to 38.7 mg/kg bw/day. Thus, the experimental animal data do not support the biological plausibility with respect to ATR exposure adversely affecting development at low exposure levels.

The regulatory ATR concentration for drinking water (US EPA 3 μg/l), which is not based on developmental toxicity endpoints, results in permissible oral ATR exposures that are ~5 orders of magnitude lower than the developmental effect levels identified in the experimental animal studies reported here. In addition, there may be a difference in response to ATR ingestion in a distributed manner throughout the day, as in drinking water, compared to bolus dosing by gavage (Foradori et al., 2014). The animal studies provide no evidence that ATR or its metabolites will interfere with human development.

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CONFLICTS OF INTEREST

Charles Breckenridge is an employee of Syngenta Crop Protection, LLC and Anthony Scialli and John DeSesso are consultants to Syngenta Crop Protection, LLC.

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