RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Case–Control Study of Maternal Residential Atrazine Exposure and Male Genital Malformations

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Manuscript Received: 31 August 2012; Manuscript Accepted: 15 November 2012

Exposure to endocrine disrupting chemicals has been associated with risk for male genital malformations. However, residential prenatal exposure to atrazine, an endocrine disrupting pesticide, has not been evaluated. We obtained data from the Texas Birth Defects Registry for 16,433 cases with isolated male genital malformations and randomly selected, population-based controls delivered during 1999–2008. County-level estimates of atrazine exposure from the United States Geological Survey were linked to all subjects. We evaluated the relationship between estimated maternal residential atrazine exposure and risk for male genital malformations in offspring. Separate unconditional logistic regression analyses were conducted for hypospadias, cryptorchidism, and small penis. We observed modest, but consistent, associations between medium-low and/or medium levels of estimated periconceptional maternal residential atrazine exposure and every male genital malformation category evaluated (e.g., adjusted odds ratio for medium compared to low atrazine levels and all male genital malformations: 1.2, 95% confidence interval: 1.1–1.3). Previous literature from animal and epidemiological studies supports our findings. Our results provide further evidence of a suspected teratogenic role of atrazine. © 2013 Wiley Periodicals, Inc.

Key words: atrazine; congenital malformations; epidemiology; hypospadias; cryptorchidism

How to Cite this Article:
Agopian AJ, Lupo PJ, Canfield MA, Langlois PH. 2013. Case–control study of maternal residential atrazine exposure and male genital malformations.
Am J Med Genet Part A 161A:977–982.

INTRODUCTION

Some of the most common male genital malformations include hypospadias (involving the urethra opening on the underside of the penis rather than the end), cryptorchidism (unilateral or bilateral undescended testes), and small penis (i.e., hypoplastic penis or micropenis). The observation that the prevalence of these defects has been increasing over the past several decades has led to the investigation of endocrine disrupting pollutants (i.e., pollutants that interfere with hormone availability or function), such as pesticides, as potential risk factors [Main et al., 2010]. Several studies have reported on associations between maternal or paternal occupational exposure to pesticides or endocrine disrupting chemicals and risk for male genital malformations in offspring [reviewed in Gaspari et al., 2011; Carmichael et al., 2012]; however, to our knowledge, residential maternal exposure to atrazine and risk for specific male genital malformations has not been studied.

Evaluating specific pesticides, such as atrazine, may be worthwhile because different pesticides may involve different (if any) teratogenic mechanisms [Gray and Ostby, 1998]. Residential exposure may be particularly relevant because after application, atrazine may enter the air, wash into surrounding soil and waterways, or migrate to groundwater [Agency for Toxic Substances and Disease Registry, 2003]. Therefore, people who live near areas of application may be exposed via air, dirt contact, and drinking water, even if they do not have direct occupational exposure [Agency for Toxic Substances and Disease Registry, 2003].

Atrazine is a good candidate pesticide to evaluate for male genital malformations because it is a potent endocrine disruptor and is the most widely used herbicide in the United States. Further, teratogenic effects of atrazine have been suggested for other types of birth defects [Munger et al., 1992; Agency for Toxic Substances and Disease Registry, 2003; Hayes et al., 2006; Winchester et al., 2009; Agopian et al., 2012]. Thus, we evaluated the relationship between estimated residential maternal exposure to atrazine during preg-

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Article first published online in Wiley Online Library (wileyonlinelibrary.com): 13 March 2013
DOI 10.1002/ajmg.a.35815

nancy and risk for male genital malformations in offspring in Texas from 1999 through 2008.

MATERIALS AND METHODS

Study Subjects

The data analyzed in this study were obtained from the Texas Birth Defects Registry. The Registry is an ongoing, population-based registry that uses active case surveillance at birthing centers, hospitals, and midwife facilities throughout Texas. The Registry is maintained by the Texas Department of State Health Services Birth Defects Epidemiology and Surveillance Branch (BDESB).

All cases in the Registry have a structural birth defect or chromosome abnormality identified within 1 year of delivery and a mother that resided in Texas at the time of delivery. Cases include live births, still births, and induced pregnancy terminations. Registry staff members identify cases and abstract medical records data for each case, which are reviewed by clinical geneticists. All case diagnoses are assigned a modified British Pediatric Association (BPA) code [National Center on Birth Defects and Developmental Disabilities, 2002]. Case records are linked to birth and fetal death certificates from the Vital Statistics Unit of the Texas Department of State Health Services.

Because testes normally descend during late pregnancy, and because, among boys with cryptorchidism at birth, spontaneous testicular descent by 6 months of age is more likely in cases with unilateral (7%) than bilateral cryptorchidism (1%) [Ferlin et al., 2008], not all potential cryptorchidism cases are coded as such in the Registry. Specifically, cryptorchidism diagnoses at $<$36 weeks gestation are only coded as such when another reportable defect is present (bilateral or laterality unspecified only) or when there has been a medical intervention for cryptorchidism. For diagnoses at $\geq$36 weeks gestation, diagnoses are coded for all bilateral or laterality unspecified cases, but only for unilateral cases when there has been another reportable defect or when there has been a medical intervention for cryptorchidism.

Unmatched controls were randomly selected among all live born infants without major malformations delivered in Texas during the study period. Reproductive and sociodemographic data was obtained from vital records for cases and controls, including data on: maternal address at delivery, delivery date, infant sex, and maternal age, race/ethnicity, birthplace, education, history of live births, and smoking (yes vs. no).

The analyses were limited to cases with documented postnatal diagnoses of a male genital malformation (BPA code: 752) and sub-analyses were conducted among cases with hypospadias (BPA codes: 752.600–752.607, 752.620, 752.625–752.627), small penis (BPA code: 752.865), or cryptorchidism (BPA codes: 752.500–752.520). Controls were selected based on a ratio of one control to one case (i.e., cases with any male genital malformation). The analyses were restricted to male cases and controls. To limit heterogeneity among cases, our analyses only included isolated cases (i.e., cases without chromosome abnormalities, malformation syndromes or sequences, or additional major birth defects, as defined by the National Birth Defects Prevention Study) [Rasmussen et al., 2003]. The protocol for this study was approved by the Institutional Review Board for the University of Texas Health Science Center at Houston.

Exposure Assessment

We obtained data for annual estimates of atrazine application levels for all Texas counties from 1999 through 2007 from the United States Geological Survey (USGS) [US Geological Survey, 2010]. The methods used to develop these exposure estimates have been previously reported in detail [US Geological Survey, 2010]. Briefly, the estimates were determined based on pesticide use data from AgroTrak surveys and data on the acreage of harvested crop from the Census of Agriculture and National Agriculture Statistics Service. The USGS estimated atrazine application levels based on agricultural crop types and acreages for those crops for which atrazine is typically applied. We have previously presented a map of USGS county-level estimates of atrazine application during 1997–2007 [Agopian et al., 2012].

For cases and controls, we linked these county-level estimated atrazine exposure data to maternal county of residence at delivery and year of conception. Because the 2008 data for atrazine exposure were not available from the USGS, we used 2007 atrazine data for 2008 deliveries. Atrazine exposure categories were assigned to all subjects, based on the distribution in controls; that is, low: below the 25th centile, medium-low: above the 25th and less than the 75th centile, medium: above the 75th and less than the 90th centile, and high: above the 90th centile, as suggested by Reynolds et al. [2003].

Statistical Analysis

For each of the male genital malformation phenotypes, we determined the count and frequency of cases. We also determined case and control counts and frequencies by maternal and infant characteristics. The count and frequency of cases and controls within each atrazine exposure category was determined (i.e., exposure categories were based on the 25th, 75th, and 90th centile cutoffs in controls). We used unconditional logistic regression to evaluate the relationship between estimated maternal residential atrazine exposure and male genital malformations. These analyses were evaluated separately for hypospadias, cryptorchidism, and small penis. Because many male genital malformations are thought to have etiological similarities [Sultan et al., 2001; Main et al., 2010; Gaspari et al., 2011], and because endocrine disrupting chemicals have been linked to broad categories of male genital malformations, such as "urogenital defects" [Munger et al., 1992; Swan, 2000; Winchester et al., 2009], we also conducted analyses among cases with any male genital defect (i.e., all cases). Further, we conducted analyses among cases with second or third degree hypospadias (i.e., urethral opening on the shaft or perineum, as opposed to the glans).

For each atrazine exposure category, we estimated crude odds ratios (ORs), using the low atrazine exposure category as the reference group. Adjusted analyses were also conducted, adjusting for the following a priori potential confounders: season of conception, birth year, and maternal age, race/ethnicity, education, history of previous live births, birthplace, and smoking. Analyses were performed using SAS (version 9.2 copyright 2002–2008, SAS, Inc., Cary, NC).

TABLE I. Frequency and Definition of Male Genital Malformations in Texas, 1999–2008

Defect	BPA[a] codes	N	%[b]
All male genital malformations	752	24,001	—
Syndromic[c] male genital malformations	752	4,268	—
Nonsyndromic male genital malformations	752	19,733	—
Isolated male genital malformations	752	16,433	(100.0)
Hypospadias	752.600–752.607, 752.620, 752.625–752.627	8,909	(54.2)
Second or third degree hypospadias	752.606, 752.607, 752.626, 752.627	738	(4.5)
Small Penis	752.865	670	(4.1)
Cryptorchidism	752.500–752.520	4,324	(26.3)

[a]Modified British Pediatric Association code.
[b]Denominator is isolated male genital malformations.
[c]Cases with chromosome abnormalities, malformation syndromes, or sequences.

RESULTS

There were 24,001 cases (19,733 nonsyndromic) with male genital malformations from 1999 through 2008 (Table I). Of these, there were 16,433 cases with isolated male genital malformations, and analyses were restricted to these cases to limit heterogeneity among cases. Thus, 16,433 controls were randomly selected among all live births without birth defects in Texas during the study period. A small proportion of cases and controls (0.9% and 1.0%, respectively) were missing data for maternal county of residence at delivery, and estimated residential atrazine exposure level could not be determined for these subjects. Of cases with cryptorchidism, 40.9% were unilateral, 57.8% were bilateral, and 1.2% did not specify laterality (data not shown).

The distributions of maternal and infant characteristics of cases with any male genital malformation and controls are presented (Table II). We observed significant differences in the distribution of delivery year, and maternal race/ethnicity, birthplace, age at delivery, education, and previous live births.

Estimated residential atrazine exposure was categorized based on levels in controls at the <25th, 25th to <75th, 75th to <90th centiles and got designated as low, medium-low, medium, and high, respectively. This resulted in the following atrazine exposure categories: low (0 to <1.55 pounds/square mile), medium-low (1.55 to <17.25 pounds/square mile), medium (17.25 to <51.90 pounds/square mile), and high (≥51.90 pounds/square mile).

In crude analyses, women with medium-low or medium levels of residential atrazine exposure were at a significantly increased risk of having offspring with any male genital malformation (crude OR: 1.18, 95% CI: 1.12–1.25 and crude OR: 1.23, 95% CI: 1.15–1.32, respectively), compared to those with low levels (Table III). Those with high levels of exposure were at a significantly decreased risk (crude OR: 0.80, 95% CI: 0.74–0.88), compared to those with low levels. The statistically significant associations with medium-low and medium exposure levels remained after adjustment for several a priori confounders (season of conception, birth year, and maternal age, race/ethnicity, education, history of previous live births, birthplace, and smoking).

Similar crude and adjusted results (i.e., consistent with an inverted U-shaped curve) were observed for hypospadias, second or third degree hypospadias, small penis, and cryptorchidism, with significant or borderline-significant associations between medium-low or medium residential atrazine exposure compared to low levels and risk for each malformation in crude and adjusted analyses (Table III). These effects were more modest (and of borderline significance) for overall hypospadias (e.g., adjusted OR: 1.07, 95% CI: 1.00–1.15 for medium-low compared to low exposure), but more pronounced for the subset with second or third degree hypospadias (e.g., adjusted OR: 1.44, 95% CI: 1.17–1.77 for medium-low compared to low exposure).

Significant associations with high compared to low levels of exposure were not observed for any of these male genital malformation categories after adjustment. To assess the possibility that this finding was due to high levels of exposure resulting in increased risk for multiple congenital malformations, post hoc crude and adjusted analyses were repeated among the 3,300 nonsyndromic cases with any male genital malformation and additional major birth defects (as opposed to the isolated cases in main analyses, which were nonsyndromic cases without additional major birth defects). Crude and adjusted results for medium-low and medium exposure were similar to the main results (Supplemental Table I); however, significant crude and adjusted associations between high compared to low exposure levels and risk for male genital defects were also observed (crude OR: 1.18, 95% CI: 1.02–1.36; adjusted OR: 1.27, 95% CI: 1.09–1.48). Due to the relatively small number of cases with multiple major birth defects, similar analyses were not conducted for specific types of male genital malformations.

DISCUSSION

We observed modest associations between medium-low and/or medium levels of estimated periconceptional maternal residential atrazine exposure and hypospadias, second or third degree hypospadias, cryptorchidism, and small penis after adjustment for several potential confounders. Similar associations were observed when we evaluated these male genital malformations separately or when evaluating all male genital malformations. We did not observe adjusted associations with high compared to low levels of exposure. These findings are consistent with an inverted U-shaped risk curve; however, the null results for high levels of exposure may reflect high

TABLE II. Characteristics of Cases With Isolated Male Genital Malformations and Controls in Texas, 1999–2008

	Cases		Controls		
Characteristic	**N**	**%**	**N**	**%**	***P*-value**
Delivery year					
1999	1,414	8.6	1,520	9.3	<0.01
2000	1,496	9.1	1,631	9.9	
2001	1,424	8.7	1,600	9.7	
2002	1,465	8.9	1,692	10.3	
2003	1,601	9.7	1,663	10.1	
2004	1,694	10.3	1,742	10.6	
2005	1,690	10.3	1,604	9.8	
2006	1,833	11.2	1,689	10.3	
2007	1,892	11.5	1,626	9.9	
2008	1,924	11.7	1,666	10.1	
Maternal race/ethnicity					
Non-Hispanic white	7,939	48.4	6,055	36.9	<0.01
Non-Hispanic black	2,136	13.0	1,848	11.3	
Hispanic	5,671	34.6	7,898	48.1	
Other	669	4.1	618	3.8	
Maternal birthplace					
United States	12,689	77.2	11,467	69.8	<0.01
Outside United States	3,744	22.8	4,966	30.2	
Maternal age					
<20	2,060	12.5	2,329	14.2	<0.01
20–24	4,205	25.6	4,589	27.9	
25–29	4,510	27.5	4,351	26.5	
30–34	3,492	21.3	3,370	20.5	
35–39	1,796	10.9	1,508	9.2	
≥40	369	2.3	285	1.7	
Maternal education					
<High school	3,756	23.1	5,131	31.6	<0.01
High school	4,742	29.2	4,792	29.5	
>High school	7,772	47.8	6,317	38.9	
Previous live births					
No	7,234	45.0	6,227	38.9	<0.01
Yes	8,836	55.0	9,793	61.1	
Maternal smoking					
No	15,341	93.7	15,365	94.0	0.42
Yes	1,025	6.3	989	6.1	
Season of conception					
Spring	4,083	24.9	4,010	24.5	0.21
Summer	4,052	24.8	3,997	24.4	
Fall	4,169	25.5	4,126	25.2	
Winter	4,071	24.9	4,236	25.9	
Total	16,433	100.0	16,433	100.0	

exposure resulting in a more severe phenotype (i.e., multiple anomalies), as discussed below.

The associations we observed were modest, but consistent across male genital malformations, which is in line with previous observations that there may be etiological similarities between these phenotypes [Sultan et al., 2001; Main et al., 2010; Gaspari et al., 2011]. Non-traditional dose–response curves (e.g., inverted U-shaped curves) have been previously associated with exposure to endocrine disrupting chemicals [vom Saal et al., 2007]. One hypothesis that could explain why an association with high atrazine levels was not observed is that high levels might result in early pregnancy loss in embryos that would have otherwise been cases. This hypothesis is supported by the observations that, in rats, embryotoxic effects of atrazine without severe maternal toxicity have been observed [Diamanti-Kandarakis et al., 2009], and that atrazine exposure has been associated with a broad range of types of birth defects in humans [Munger et al., 1992; Winchester et al., 2009; Waller et al., 2010; Agopian et al., 2012]. Further, in post hoc analyses repeated among nonsyndromic cases with additional malformations, we observed significant associations between high versus low atrazine exposure and risk for male genital defects. This finding seems consistent with the hypothesis that, in some embryos, high levels may lead to a more severe phenotype than isolated male genital defects. The exact mechanisms involved are unclear and further research is needed to confirm and explain our findings.

Previous studies support the plausibility of an association between atrazine exposure and male genital malformations. Studies in fish, amphibians, reptiles, and mammals have demonstrated that atrazine demasculinizes male gonads [reviewed in Hayes et al., 2011]. Further, atrazine has been shown to induce hypospadias, specifically, in rats [Diamanti-Kandarakis et al., 2009]. Although the exact mechanisms by which endocrine disruptors may lead to defects of male genitalia are unknown, it is thought that these chemicals may interfere with key processes that lead to male sexual differentiation. For instance, as male genital development depends on the conversion of testosterone to 5α-dihydrotestosterone or estradiol within target tissues through the enzymatic activity of 5α-reductase or aromatase, endocrine disruptors such as atrazine, may block the activity of these enzymes, thereby affecting the process of development [Schug et al., 2011].

To our knowledge, three epidemiological studies have evaluated atrazine and male urogenital defects. Munger et al. evaluated statewide ecological (i.e., not individual-level) data in Iowa and reported an excess of urogenital defects (unadjusted relative risk: 3.5, 95% CI: 2.2–5.3) in communities served by a water supply system with elevated atrazine levels compared to other communities [Munger et al., 1992]. Winchester et al. evaluated United States Centers for Disease Control and Prevention natality data sets, and observed a significantly higher prevalence of "other urogenital anomalies," but not "malformed genitalia," among infants conceived during months with elevated concentrations of atrazine in surface water [Winchester et al., 2009]. However, Meyer et al. evaluated and estimated atrazine exposure (based on proximity to agricultural application sites) and hypospadias in eastern Arkansas and found no association [Meyer et al., 2006]. Associations between atrazine and other birth defects have also been reported, including congenital heart defects, spina bifida, gastroschisis, cleft lip, choanal atresia, and limb reduction defects [Munger et al., 1992; Agency for Toxic Substances and Disease Registry, 2003; Winchester et al., 2009; Waller et al., 2010; Agopian et al., 2012]. Our findings further support the suspected notion that atrazine may have broad teratogenic effects.

Our results should be interpreted in consideration of certain limitations. Because large-scale population-based individual meas-

TABLE III. Association Between Atrazine and Isolated Male Genital Malformations in Texas, 1999–2008

Atrazine levels[a]	Pounds/square mile	Cases (%)	Controls (%) (N = 16,433)	OR	95% CI	aOR[b]	95% CI
All male genital malformations (N = 16,433)							
Low (reference)	0 to <1.55	3,546 (21.8)	3,918 (24.1)	1.00		1.00	
Medium-low	1.55 to <17.25	8,849 (54.4)	8,295 (51.0)	**1.18**	**1.12–1.25**	**1.19**	**1.12–1.26**
Medium	17.25 to <51.90	2,680 (16.5)	2,402 (14.8)	**1.23**	**1.15–1.32**	**1.20**	**1.11–1.29**
High	≥51.90	1,207 (7.4)	1,660 (10.2)	**0.80**	**0.74–0.88**	0.96	0.87–1.05
Hypospadias (N = 8,909)							
Low (reference)	0 to <1.55	1,989 (22.5)	3,918 (24.1)	1.00		1.00	
Medium-low	1.55 to <17.25	4,659 (52.8)	8,295 (51.0)	**1.11**	**1.04–1.18**	1.07	1.00–1.15
Medium	17.25 to <51.90	1,485 (38.2)	2,402 (14.8)	**1.22**	**1.12–1.33**	1.09	1.00–1.20
High	≥51.90	691 (7.8)	1,660 (10.2)	**0.82**	**0.74–0.91**	1.00	0.89–1.11
Second or third degree hypospadias (N = 738)							
Low (reference)	0 to <1.55	133 (18.2)	3,918 (24.1)	1.00		1.00	
Medium-low	1.55 to <17.25	428 (58.6)	8,295 (51.0)	**1.52**	**1.25–1.85**	**1.44**	**1.17–1.77**
Medium	17.25 to <51.90	117 (16.0)	2,402 (14.8)	**1.44**	**1.11–1.85**	1.18	0.90–1.55
High	≥51.90	53 (7.3)	1,660 (10.2)	0.94	0.68–1.30	1.00	0.71–1.41
Small penis (N = 670)							
Low (reference)	0 to <1.55	152 (23.1)	3,918 (24.1)	1.00		1.00	
Medium-low	1.55 to <17.25	334 (50.8)	8,295 (51.0)	1.04	0.85–1.26	1.05	0.86–1.28
Medium	17.25 to <51.90	126 (19.2)	2,402 (14.8)	**1.35**	**1.06–1.72**	**1.38**	**1.07–1.77**
High	≥51.90	45 (6.9)	1,660 (10.2)	**0.70**	**0.50–0.98**	0.74	0.52–1.04
Cryptorchidism (N = 4,324)							
Low (reference)	0 to <1.55	997 (22.8)	3,918 (24.1)	1.00		1.00	
Medium-low	1.55 to <17.25	2,331 (54.5)	8,295 (51.0)	**1.13**	**1.04–1.23**	**1.17**	**1.08–1.28**
Medium	17.25 to <51.90	621 (14.5)	2,402 (14.8)	1.04	0.93–1.16	**1.14**	**1.01–1.28**
High	≥51.90	348 (8.1)	1,660 (10.2)	**0.84**	**0.73–0.96**	0.93	0.80–1.07

Bold indicates statistical significance.
[a]Atrazine categories based on 25th, 75th, and 90th centiles in controls.
[b]Odds ratio adjusted for season of conception, birth year, and maternal race/ethnicity, education, age, history of previous live births, birthplace, and smoking.

urements of periconceptional atrazine exposure are not available (and would be difficult to collect for rare outcomes such as birth defects), we used county-level estimates of atrazine exposure, which may introduce the potential for exposure misclassification. However, personal atrazine exposure has been shown to be highly correlated with living in areas with high levels of atrazine application [Agency for Toxic Substances and Disease Registry, 2003; Hayes et al., 2006; Curwin et al., 2007]. Additionally, because maternal residence at conception was not available, we used residence at delivery as a proxy. It has previously been shown that residential mobility during pregnancy is not expected to result in a meaningful change in county-based exposure assessment in this population [Lupo et al., 2010]. County of residence at conception and delivery are expected to be the same for >96% of cases and controls in Texas and any residential mobility outside of a given county is expected to occur at similar proportions between cases and controls [Lupo et al., 2010]. Although atrazine is the most widely used pesticide in the United States, we also cannot rule out the possibility that the observed associations are due to another pesticide that is correlated with atrazine use. We also cannot rule out the possibility that some case misclassification may have occurred (e.g., over- or under-diagnosis of cryptorchidism).

This study also had several strengths. We used a large, population-based sample with active surveillance to identify cases. This data set includes cases that were stillbirths and elective pregnancy terminations, whereas data sets that do not include non-live born cases are more susceptible to potential selection bias. Further, we limited main analyses to cases with isolated male genital malformations, which likely reduced etiological heterogeneity among cases.

In summary, we report on modest, but consistent, inverted U-shaped associations between estimated maternal residential exposure to atrazine and several genital malformations in male offspring. Our results add to a growing body of literature suggesting teratogenic effects of atrazine on the developing male reproductive system and on other systems of the body. However, the direction of the observed associations (i.e., inverted U-shaped) may suggest that potential teratogenic effects on male genitalia occur via a different mechanism than other types of birth defects (i.e., those with a suspected monotonic relationship with atrazine). Further research is needed to confirm our findings and to better understand the mechanisms involved.

ACKNOWLEDGMENTS

The authors thank the staff of the Birth Defects Epidemiology and Surveillance Branch, Texas Department of State Health Services, for help with collecting data for the Texas Birth Defects Registry. This

project was supported in part by the Centers for Disease Control and Prevention (CDC)-funded Texas Center for Birth Defects Research and Prevention (#5U01DD000494) through a cooperative agreement with Texas Department of State Health Services (DSHS), as well as the Title V Office of Texas DSHS. The contents of this study are solely the responsibility of the authors and do not necessarily represent the official view of the CDC.

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