Committee for Risk Assessment

RAC

Annex 3

Records

of the targeted public consultation on the reproductive toxicity of

Acetochlor (ISO); 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide

EC number: 251-899-3

CAS number: 34256-82-1

CLH-O-0000001412-86-26/F

The proposal for the harmonised classification and labelling (CLH) of acetochlor was submitted by Spain in September 2013; it was subject to public consultation from 5 December 2013 until 20 January 2014. The comments received by that date are compiled in Annex 2 to this opinion.

After the closure of the public consultation, the Committee for Risk Assessment (RAC) noted that the data from the repeated dose toxicity studies as well as from the reproductive toxicity studies indicated that classification for reproductive toxicity should be considered for acetochlor .

As the Dossier Submitter initially did not propose classification for reproductive toxicity, and in order to strengthen the information base, ECHA launched an additional public consultation focussing on the potential reproductive toxicity of acetochlor. The consultation started on 18 September 2014 and finished on 6 October 2014. The comments received are compiled in this annex.

COMMENTS AND RESPONSE TO COMMENTS RECEIVED DURING THE TARGETED CONSULTATION (18 SEPTEMBER – 6 OCTOBER 2014)

Substance name: Acetochlor (ISO); 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide

CAS number: 34256-82-1

EC number: 251-899-3

Date	Country / Person / Organisation / MSCA	Comment	RAC response to comment
06/10/2014	French CA	France is of the opinion that a classification of acetochlor for fertility effects is not warranted. Effects on fertility observed in the 2-generation study in rats comprised decrease in the number of implantations in F0 and F1 females and in the number of live pups on postnatal day 1 in F2 litters at the high dose level of 1750 ppm. These effects occurred in the presence of parental toxicity. Testicular effects (organ weight and histopathological observations) were observed in the dog studies. Histopathological changes are confined to the high dose group of the 1-year study (50 mg/kg bw/d) according to the histopathology review performed in 2003. In this group, males showed clinical signs of toxicity with severe neurological changes, associated with decreased bodyweight and food consumption. Moreover 2 out of 5 males were killed prematurely in this group. Thus, the effects on testes may be related to this poor health conditions which could have delayed the puberty. Furthermore, no effects on testes were observed in rats and mice in repeated dose studies. If effects on male reproductive organs would be responsible for the fertility effects observed in the 2-generation rat study, this means that a high reduction in sperm count of more	Thank you for your comments. The issue of background systemic toxicity at high doses is important and makes it difficult to identify specific toxicity associated with Acetochlor exposure. A good argument can be made for chronic renal failure and this may be associated with the testicular effects in dogs. However there is no confirmatory data that the dogs are indeed in chronic renal failure or that the testicular effects are secondary to the generalised toxicity that is apparent in these animals. RAC disagrees with the idea that effects on testes should have been observed in rat repeated dose studies at similar dose levels simply because the dog is a more

Date	Country / Person / Organisation / MSCA	Comment	RAC response to comment
		than 90% would be observed in rats (rats are still fertile after a reduction of sperm count up to around 90%). Therefore, effects on testes should have been observed in rat repeated dose studies at similar dose levels. As a conclusion, based on the available data, there is no convincing evidence that acetochlor should be classified for fertility effects.	sensitive species and shows effects at much lower doses than in rats.
06/10/2014	Monsanto	Monsanto is grateful to have been given the opportunity to comment on the proposed classification for reproductive toxicity of acetochlor, and our comments are below.	Thank you for your comments and a well structured and argued document. You make many clear points and these are represented in the finalised opinion document. The reproductive toxicity proposal was not in the original CLH report but arose from the considerations of RAC. It is unclear as to what extra useful data can originate from a further evaluation of the histological slides from the four dog studies. The Creasy (2003) report is recognised as a valuable contribution to these discussions and is considered fairly in the ODD. The remaining issue that a re-evaluation is unlikely to change is the severity of the testes pathology in the high dose animals from the two 1-year studies. A

Date	Country / Person / Organisation / MSCA	Comment	RAC response to comment
		Monsanto Company Response to the Proposal by ECHA to Classify Acetochlor as Category 2 for Reproductive Toxicity 6 October 2014 The RAC proposed classification of acetochlor as a Category 2 reproductive toxicant based on 3 endpoints of reproductive toxicity which are considered to be of concern, i.e. testicular toxicity in the dog after one year of treatment, decrease in rat ovary weight, and a decrease in the number of implantations in a rat multigeneration reproductive toxicity study. Monsanto has reviewed the entire acetochlor toxicology database to better understand the meaning of these findings and to investigate whether they should be considered as a basis for the classification of acetochlor as a reproductive toxicant. Our position is summarized below. In addition, we have included a few specific comments concerning the discussion of reproductive toxicity in the 2nd draft of the RAC CLH Opinion Development Document (ODD) and the presentation by the rapporteur during RAC-30. **Testicular toxicity in the dog** ***Study Results*** In a 12-month repeated dose toxicology study in the dog (Broadmeadow, 1989), a decrease in absolute (48%) and relative (34%) testis weight was noted at 50 mg/kg bw/day. Associated histopathological findings were slight to moderate tubular degeneration (5/5), moderate to marked maturation arrest (5/5) and slight to moderate sperm giant cells (4/5) in the testes, and moderate to severe hypospermia (5/5) in the epididymides. These testicular changes were believed to have occurred during the latter half of the study. Progressive weight loss (emaciation) and severe neurobehavioural clinical signs were also noted at this dose level, resulting in the killing of 2/5 male and 4/5 female dogs for humane reasons between weeks 39 and 51. Animals in the 50 mg/kg bw/day group also exhibited severe renal toxicity as indicated by histopathology findings and marked increases in blood urea (80%) and creatinine (50%). There was no treatment-related effect on testicular weight at 10 mg/kg bw/day but moderate (1/5, dog 1360) and minimal to slight (3/5) testicular tubular degeneration were reported at this dose level by the original Study Pathologist. A sperm granuloma was also noted in 1/5 dogs (dog 1360). Hypospermia was marked to severe in both epididymides in dog 1360 and slight in the right epididymis only of another dog at this dose level. Renal toxicity was also present at 10 mg/kg bw/day but less prominent than at 50 mg/kg bw/day and without a change in blood urea and creatinine. Page 1 of 7	histopathological review is unlikely to shed clarity on the mechanism for the clear effects noted in the high dose animals. However, the arguments have been given due consideration.

Histopathology peer review of the testes and epididymides slides from all males in the Broadmeadow (1989) study was subsequently conducted by Dr. Dianne Creasy, a leading expert in testicular pathology. Dr. Creasy confirmed the presence of treatment-related testicular lesions at 50 mg/kg bw/day but, contrary to the original Study Pathologist, concluded that no treatment-related findings were present at 10 mg/kg bw/day (Creasy, 2003). Dr. Creasy reported that the high-dose males showed partial germ cell depletion and active degeneration of germ cells, and that these effects were most prominent during the latter stages of spermatid development. Despite these findings, active spermatogenesis was still ongoing in most tubules, suggesting the effect was largely reversible. Contrary to the original findings, Dr. Creasy concluded that testicular lesions were present in only one animal (dog 1360) at 10 mg/kg bw/day. This animal exhibited slight germ cell degeneration, slight partial germ cell depletion, and slight segmental tubular atrophy associated with slight multifocal tubular necrosis with lymphocytic infiltrate, a finding not observed in any of the high-dose animals. Severe hypospermia combined with multifocal interstitial lymphocytic aggregates and germ cell debris were observed in the epididymides of dog 1360, a testicular histopathological profile that was not observed in any of the other animals in this study. Based on these findings, Dr. Creasy concluded that the testicular and epididymal lesions in dog 1360 were not related to acetochlor treatment but instead indicative of lymphocytic orchitis which is an autoimmune disease sometimes observed in beagle dogs.

In another 12-month repeated dose toxicology study in the dog (Ahmed, 1981), a decrease in absolute (51%) and relative (40%) testis weight was noted at 40 mg/kg bw/day with mild (1/6) and moderate (5/6) diffuse testicular atrophy characterized as a reduction in size of the seminiferous tubules and absence of spermatogenesis. At this dose level, a decrease in absolute body weight of 14% and a decrease of cumulative body weight gain over the entire dosing period of 47% was observed. An even greater decrease in body weight gain (75%) was observed during the last six months of the study which corresponds to the time period when the testicular effects are believed to have occurred. Kidney weight was increased by 18% but there were no histopathological changes and no increase in blood biochemistry markers indicative of renal toxicity. No testicular toxicity was observed at 12 mg/kg bw/day, which supports Dr. Creasy's conclusion that the testicular findings at 10 mg/kg bw/day in the other 1-year dog study were not related to treatment.

Discussion

Testicular toxicity following acetochlor administration was observed only in the two 1-year dog studies. No treatment-related decrease in testes weight or histopathology was observed in dog studies of 13 to 17 weeks, mouse studies of 13 weeks to 23 months, or rat studies of 13 weeks to 24 months. In addition, no treatment-related decrease in relative testes or epididymides weight or histopathology were noted in 3 multigeneration reproduction studies in the rat. No change in sperm characteristics

(number, morphology, motility) was reported in the F0 and F1 generations in the most recent multigeneration reproductive toxicology study (Milburn, 2001).

Treatment-related testicular effects in the two 1-year dog studies were elicited only in the presence of clear toxicity: severe weight loss and nephrotoxicity at 50 mg/kg bw/day in the Broadmeadow (1989) study and a substantial decrease in weight gain and kidney changes at 40 mg/kg bw/day in the Ahmed (1981) study. The histopathological changes in the kidneys of the dogs at 50 mg/kg bw/day combined with the marked increase in urea and creatinine blood levels are indicative of renal failure. Severe renal failure produces an accumulation of toxic endogenous metabolites (end products of amino acid and protein metabolism) which may have caused the severe neurobehavioral and neuropathological findings (uremic syndrome). An accumulation of toxic endogenous metabolites and a decreased renal clearance of acetochlor metabolites could also have led to altered testicular function.

Although treatment-related testicular effects were originally also reported at 10 mg/kg bw/day in the Broadmeadow (1989) study, this finding was contradicted by Dr. Creasy, a leading expert in testicular pathology. In addition, no testicular toxicity was observed at 12 mg/kg bw/day in the other 1-year dog study, which supports Dr. Creasy's conclusion. It should also be noted that formalin was used as the fixative of the testes from both studies. The use of formalin for testes histopathology examination is no longer recommended as it greatly complicates the microscopic evaluation and can lead to difficulties in interpretation.

Because of the uncertainty regarding the interpretation of the findings at 10 mg/kg bw/day, as well as the difficulties likely introduced by the use of formalin, Monsanto has arranged for the histology slides of the testes and epididymides from the 90-day, 119-day, and two 1-year dog studies to be reviewed by a formal Pathology Working Group (PWG) composed of Jerry Hardisty (chair), Dianne Creasy, Eric van Esch, Catherine Picut-Parker, and Andrew Suttie. Several of these pathologists are considered experts in the histopathology of reproductive organs. The PWG evaluation will take place in late November, with a final report likely to be issued by end-January or early February. The results of this PWG are critical for a proper understanding of the potential for acetochlor to induce testicular toxicity in dogs. Therefore, we respectfully request that a final decision as to whether or not to classify acetochlor as Category 2 for reproductive toxicity be deferred until the results of the PWG are available for RAC review.

Acetochlor ovarian toxicity in the rat

In the most recent multigeneration study in the rat (Milburn, 2001), statistically significant decreases in absolute (25% for F0, 22% for F1) and relative (12% for F0, 10% for F1) ovary weights were observed at 1750 mg/kg diet (ppm) corresponding with approximately 200 mg/kg bw/day. However,

there were no treatment-related effects on ovarian histopathology or fertility. The effect on ovary weight was limited to the high-dose level animals and occurred in the presence of other toxicity, such as significant decreases in body weight (10% for F0, 14% for F1) and body weight gain (19% for F0, 11% for F1) during the pre-mating period, and increases in liver, kidney and thyroid weights, and nasal proliferative lesions. It should also be noted that the 1750 mg/kg diet (ppm) dose level is above the Maximum Tolerated Dose for chronic exposure which is approximately 1000 mg/kg diet (ppm).

No treatment-related decrease in absolute and relative ovary weight and no treatment-related changes in the histopathology of the ovaries, or mating and fertility indices were observed in a second multigeneration study in the rat (Willoughby, 1989) at the same dietary level (1750 mg/kg diet). The ODD indicated that this study cannot be fully relied on since it had significant deficiencies. However, this study fully complied with the OECD and USEPA guidelines then in effect. The reason it was considered deficient was that it did not include the newer endpoints added by the 2001 OECD guidelines (e.g. sperm parameters and developmental milestones). The absence of those parameters has no impact on the validity of the remaining data, such as ovarian weight, fertility, number of implants, litter size, etc.).

A decrease in absolute ovary weight (35%) in the F1 generation was reported in an older multigeneration study (Schardein, 1982) at a dietary concentration of 5000 mg/kg diet (ppm), a dose level which also induced a marked decrease in body weight (25% for F0 at 34 weeks, 33% for F1 at 74 weeks) as well as decreased food consumption and renal toxicity. There were no effects on relative ovary weight, ovarian histopathology or fertility.

No treatment-related decrease in absolute or relative ovary weight or histopathological changes in the ovaries were noted in two 13-week and one 24-month studies in the rat. Treatment-related decreases in absolute ovary weight but not in relative ovary weight were observed in the two other 24-month studies in the rat but were not accompanied by histopathological changes and were thus considered a result of decreased body weights.

Thus, the decrease in ovary weight observed in one study in the presence of significant general maternal toxicity is not considered indicative of a specific reproductive effect or relevant for classification.

Acetochlor effect on implantations and litter size in rats

Treatment-related decreases in the number of implants (11% for F0, 13% for F1), the number of live pups (16% for F1), and the number of live and dead pups (10% for F0 and 16% for F1) was found at 1750 mg/kg diet (ppm) in the most recent multigeneration study in the rat (Milburn, 2001). However,

as indicated above, this occurred only in the presence of significant maternal toxicity, including a decrease in body weight (10% for F0, 14% for F1) and body weight gain (19% for F0, 11% for F1) during the pre-mating period, increases in liver, kidney and thyroid weights, and nasal proliferative lesions.

No treatment-related effect was observed on litter size at the same dietary level (1750 mg/kg diet) in a second multigeneration study in the rat (Willoughby, 1989). In addition, no treatment-related effect was found on the number of viable fetuses/dam, number of implants/dam and number of corpora lutea/dam in 2 embryo-fetal toxicology studies each in the rat and the rabbit. Implantation loss was seen in one rat developmental toxicity study, but only at 600 mg/kg bw/day which is a dose causing severe maternal toxicity such as mortality (2/25), marked decrease in body weight gain (62% at GD 6-7, 153% at GD 7-9, 26% at GD 9-12, 38% of adjusted body weight at GD 6-20) and food consumption, clinical signs of toxicity and a marked increase in water consumption.

Thus, the decrease in the number of implants and litter size in one multigeneration study in the presence of significant general maternal toxicity is not considered indicative of a specific reproductive effect or relevant for classification.

SUMMARY AND CONCLUSION

The weight of evidence indicates that acetochlor has only minimal potential to induce reproductive toxicity and that such effects occur only in the presence of significant other toxicity.

- Three multi-generation rat reproduction toxicology studies have been conducted with acetochlor. Although slight effects on several reproductive endpoints (e.g. decrease in the number of implants and litter size) were noted in the most recent study, these occurred only at a dose level that caused significant maternal toxicity. No such reproductive effects were noted at similar dose levels in either of the two other rat reproduction studies. These older two studies did not include the newer endpoints required by the 2001 OECD guidelines but were fully adequate to assess the reproductive endpoints in question for acetochlor.
- Although decreases in absolute ovarian weights were observed in several rat studies, this generally was a result of decreased body weights. Except for a slight decrease at the high-dose level in the Milburn reproduction study, there were no meaningful, dose-related decreases in relative ovarian weights and no treatment-related ovarian histopathological lesions in any of the rat, mouse or dog studies with acetochlor.
- No evidence of testicular toxicity was noted in any of the studies with rats or mice, or in the 90-day or 119-day studies with dogs.
- Clear evidence of testicular toxicity (decreased testes weights and testicular histopathology) was noted in two 1-year dog studies. However, definitive effects occurred only at dose levels

(50 and 40 mg/kg bw/day) that caused significant general toxicity, including weight loss, severe renal toxicity and loss of 6/10 animals in one study at 50 mg/kg bw/day and lesser effects in the other study at 40 mg/kg bw/day. Initially, histopathological lesions of the testes were also reported at 10 mg/kg bw/day in one of the 1-year dog studies. However, this finding was contradicted by a subsequent peer review of the slides by Dr. Creasy, a leading expert in testicular pathology. Dr. Creasy concluded that testicular lesions were present in only one dog at 10 mg/kg bw/day and that these lesions were different in nature than those seen in the high-dose dogs. Dr. Creasy also concluded that the lesions at 10 mg/kg bw/day were not related to treatment with acetochlor but were instead caused by lymphocytic orchitis, an autoimmune disease sometimes seen in beagle dogs. Dr. Creasy's conclusion is supported by the fact that no testicular lesions were noted at 12 mg/kg bw/day in the other 1-year dog study.

Based on the above information, we believe that the weight of evidence does not support classification of acetochlor as a reproductive toxicant. However, because of the differing conclusions regarding the effects seen in the 10 mg/kg bw/day dogs, and the fact that the use of formalin for preservation of the testes likely contributed to difficulties in interpretation, Monsanto has authorized a formal Pathology Working Group to evaluate the testes and epididymides from all four of the dog studies mentioned (90-day, 119-day and two 1-year dog studies). Preliminary results from this PWG should be available in early December with a final report likely by end-January or early February 2015. The results from this evaluation are essential for a proper evaluation of the potential for acetochlor to cause testicular toxicity. Therefore, we respectfully request that the final decision as to whether or not to classify acetochlor as Category 2 for reproductive toxicity be delayed until the results from the PWG evaluation can be evaluated by the RAC.

Comments on the CLH Opinion Development Document (Draft 2) and Rapporteur Presentation

- ODD, page 46[1], RAC evaluation of repro tox, Summary of DS proposal, (1) Fertility: The table and text indicates that the Willoughby (1989) study is not acceptable due to guideline deviations and missing data for many reproductive indices. However, this study fully complied with the existing OECD guidelines at the time it was conducted. The data missing were for the new endpoints included in the 2001 OECD guidelines (e.g., sperm parameters, developmental milestones). A similar statement was made on page 51.
- ODD, top of page 49, Consideration of Category 2: The last 2 bullets indicate that there were testicular effects in dogs at 10 mg/kg bw/day but do not acknowledge that this finding was contradicted by Dr. Creasy, a leading expert in testicular pathology.

[1] The ODD page numbers listed here may not be the same on each computer due to different pagination of the Word document that was reviewed

- ODD, page 50, Supplemental Information, In depth analyses by RAC: The statement in the text that the decreased ovarian weights in the Milburn study were supported by the Schardein study does not acknowledge the fact that the dose-related differences in the Schardein study were only in absolute ovary weights, not in relative ovary weights, and were attributed to the significant decreases in body weight. The text also does not acknowledge that no differences in ovarian weight were noted in the Willoughby study.
- ODD, page 51: For completeness, we suggest including a comparable table of reproductive parameters (implantation, live pups, etc.) from the other two reproduction studies.
- Presentation, slide 20: For completeness, we believe that the slide on testicular effects in dogs should include a row indicating no testicular effects at 12 mg/kg bw/day. It would also be appropriate to include more details/rationale regarding the Creasy conclusion.
- Presentation, slide 22: For completeness, we believe that the organ weight and implantation data from the other two reproduction studies should also be shown, as well as a statement about lack of testicular and ovarian weight changes in the other rat (and mouse) studies.

Reproductive toxicity

Date	Country / Person / Organisation / MSCA	Comment	RAC response to comment
24/09/2014	R.I.S.K. (Rebutting Industry Science with Knowledge) Consultancy	Dear RAC: Given that two NGO audits of Registration dossiers show that applicants illegally review less than a quarter of published financially-independent toxicity studies, I urge you to consider the below data, directly relevant to your call for data. If it has been considered on the road to Authorization, I ask you to reconsider it with minds open to the possibility that toxicity studies performed by disinterested parties may have more worth than those sponsored by the party whose every interest is that its product be Authorized. known insensitivities of the OECD TG test methods include use of quasi-poisonous doses (thus not testing the effects of realistic chronic exposures), destroying almost all evidence of elicited chronic disease (i.e. sacrifice of animal groups at human equiv. of ~60 yrs.), historical controls and few positive controls. I also notes in my quick PubMed search ('Acetochlor toxicity') many reliable indications of thyroid mechanism of action, including cancer), and regular findings of red blood cell toxciity. **Subject: 5 selected items - PubMed** This message contains search results from the National Center for Biotechnology Information (NCBI) at the U.S. National Library of Medicine (NLM). Do not reply directly to this message Sent on: Wed Sep 24 08:20:06 2014	Thank you for your comments. Please be assured that RAC does indeed approach each assessment with an open mind and that the process endevours to seek all the available information in deciding on an outcome. While every effort is made to ascertain the available data, peer-reviewed and accessible publications do not always provide the extensive records necessary for a full evaluation with respect to the reliability of the data and the techniques used. Only two publications from the list provided (Swan et al., 2003; Ashby et al., 1997) are publicly available, with the latter providing support for the non-mutagenicity of Acetochlor in rat germ cells. The study by Swan *et al*., (2003) illustrated an inverse association between levels of pesticide residues in human urine and parameters of human semen quality except in the case for Acetochlor.

5 selected items

PubMed Results

Items 1 - 5 of 5 (Display the 5 citations in PubMed)

1 Toxicol Mech Methods. 2011 Jun;21(5):406-17. doi:
. 10.3109/15376516.2010.551554. Epub 2011 Feb 15.

NEONATAL EXPOSURE TO HERBICIDE ACETOCHLOR ALTERS PUBERTAL DEVELOPMENT IN FEMALE WISTAR RATS.

Rollerova E[1], Wsolova L, Urbancikova M.

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Abstract

• **OBJECTIVES:**

The purpose of this study was to evaluate the effects of neonatal exposure to the herbicide acetochlor (ACT) on pubertal development and reproductive functions in female Wistar rats and to investigate capability of ACT to interfere with estradiol binding to rat uterine estrogen receptors (ERs) ex vivo.

• **METHODS:**

Acetochlor (7.68 and 15.36 mg/kg/day) was administered by

		subcutaneous injection from postnatal day (PND) 4-7, and vaginal opening, and estrous cyclicity were evaluated from PND 8-159. A second group of adult ovariectomized female rats was dosed for 6 days with ACT (153.6 mg/kg/day, oral gavage). The interference of ACT with the binding of [^{3}H]Estradiol -17β to uterine nuclear and cytoplasmic estrogen receptors was analyzed ex vivo in receptor binding assay. • **RESULTS:** Both doses of ACT caused acceleration of the age at eye opening and vaginal patency that were significantly different from the control. In addition, altered estrous cyclicity was observed in the ACT (15.36 mg/kg/day) group with 54% of the female rats displaying irregular cycles at PND 159. While uterine weights were not altered, a significant accumulation of uterine nuclear estrogen receptors was observed in the ACT group. • **CONCLUSION:** These results indicate that acetochlor can act as the endocrine disruptor and that endpoints related to pubertal development and reproductive functions sensitive sites are targeted with this persistent pollutant. PMID: 21320039 [PubMed - indexed for MEDLINE] Related citations 2 Ecotoxicology. 2008 May;17(4):280-6. doi: 10.1007/s10646-008-0195-z. . Epub 2008 Feb 23.	

		EVALUATION OF DNA DAMAGE IN CHINESE TOAD (BUFO BUFO GARGARIZANS) AFTER IN VIVO EXPOSURE TO SUBLETHAL CONCENTRATIONS OF FOUR HERBICIDES USING THE COMET ASSAY. Yin XH[1], Li SN, Zhang L, Zhu GN, Zhuang HS. Author information: [1]College of Environmental Science and Engineering, Dong Hua University, Shanghai 201620, China. **Abstract** Chinese toad, Bufo bufo gargarizans, is frequently found in rice fields, muddy ponds, wetlands and other aquatic ecosystems in China. Because of its habitat, it has many chances of being exposed to pesticides, such as acetochlor, butachlor, chlorimuron-ethyl, and paraquat, which are extensively used in rice or cereal fields. Amphibians may serve as model organisms for determining the genotoxic effects of pollutants contaminating these areas. In the present study DNA damage was evaluated in the Chinese toad using the comet assay, as a potential tool for the assessment of ecogenotoxicity. The first step was to determine the acute toxicity of the above-mentioned herbicides. In acute tests, tadpoles were exposed to a series of relatively high concentrations of acetochlor, butachlor, chlorimuron-ethyl, and paraquat for 96 h. The LC(50)(96 h) of acetochlor, butachlor, chlorimuron-ethyl and paraquat were measured as 0.76, 1.32, 20.1 and 164 mg l(-1), respectively. Also, negative effects on the behavior of tadpoles were observed with acetochlor, butachlor, and paraquat. Secondly, the comet assay was used for detecting DNA damage in Chinese toad tadpoles exposed to sublethal concentrations of four herbicides. Significant ($P < 0.05$)	

		concentration-dependent increase in DNA damage (as indicated by tail length, tail moment, olive tail moment) were observed from erythrocytes of tadpoles exposed to sublethal concentrations of acetochlor, butachlor, paraquat, and methyl methanesulfonate, except chlorimuron-ethyl. To our knowledge, this is the first report describing the use of Bufo bufo gargarizans for genotoxicity assessment of herbicides. PMID: 18297398 [PubMed - indexed for MEDLINE] Related citations 3 Environ Health Perspect. 2003 Sep;111(12):1478-84. . **SEMEN QUALITY IN RELATION TO BIOMARKERS OF PESTICIDE EXPOSURE.** Swan SH[1], Kruse RL, Liu F, Barr DB, Drobnis EZ, Redmon JB, Wang C, Brazil C, Overstreet JW; Study for Future Families Research Group. Author information: [1]Department of Family and Community Medicine, MA 306 Medical Sciences Building, University of Missouri-Columbia School of Medicine, Columbia, MO 65212, USA. swans@health.missouri.edu **Comment in** • Missing Link?: Alachlor and Semen Quality. [Environ Health Perspect. 2005] **Abstract**	

		We previously reported reduced sperm concentration and motility in fertile men in a U.S. agrarian area (Columbia, MO) relative to men from U.S. urban centers (Minneapolis, MN; Los Angeles, CA; New York, NY). In the present study we address the hypothesis that pesticides currently used in agriculture in the Midwest contributed to these differences in semen quality. We selected men in whom all semen parameters (concentration, percentage sperm with normal morphology, and percentage motile sperm) were low (cases) and men in whom all semen parameters were within normal limits (controls) within Missouri and Minnesota (sample sizes of 50 and 36, respectively) and measured metabolites of eight current-use pesticides in urine samples provided at the time of semen collection. All pesticide analyses were conducted blind with respect to center and case-control status. Pesticide metabolite levels were elevated in Missouri cases, compared with controls, for the herbicides alachlor and atrazine and for the insecticide diazinon [2-isopropoxy-4-methyl-pyrimidinol (IMPY)]; for Wilcoxon rank test, $p = 0.0007$, 0.012, and 0.0004 for alachlor, atrazine, and IMPY, respectively. Men from Missouri with high levels of alachlor or IMPY were significantly more likely to be cases than were men with low levels [odds ratios (ORs) = 30.0 and 16.7 for alachlor and IMPY, respectively], as were men with atrazine levels higher than the limit of detection (OR = 11.3). The herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and metolachlor were also associated with poor semen quality in some analyses, whereas acetochlor levels were lower in cases than in controls ($p = 0.04$). No significant associations were seen for any pesticides within Minnesota, where levels of agricultural pesticides were low, or for the insect repellent DEET (N,N-diethyl-m-toluamide) or the malathion metabolite malathion dicarboxylic acid. These associations between current-use pesticides and reduced semen quality suggest that agricultural chemicals may have contributed to the reduction in semen quality in fertile men from mid-Missouri we	

		reported previously. PMCID: PMC1241650 **Free PMC Article** PMID: 12948887 [PubMed - indexed for MEDLINE] Related citations 4 Teratog Carcinog Mutagen. 2000;20(4):229-40. . **NONGENOTOXIC (EPIGENETIC) CARCINOGENS: PESTICIDES AS AN EXAMPLE. A CRITICAL REVIEW.** Rakitsky VN[1], Koblyakov VA, Turusov VS. Author information: [1]F.F. Erisman Moscow Research Institute of Hygiene, Moscow, Russia. **Abstract** The following groups of pesticides are considered in this review by supposed mechanisms of their carcinogenicity: hepatocarcinogenic pesticides, pesticides - peroxisome proliferators, pesticides as endocrine disruptors, goitrogenic pesticides, pesticides producing sustained cell proliferation and some others. With very rare exceptions, pesticides do not react with DNA directly and the mechanisms of their carcinogenicity are, in general, similar to those of other nongenotoxic (epigenetic) carcinogens, namely: promotion of spontaneous initiation, cytotoxicity with sustained cell proliferation, oxidative stress, formation of activated receptors and some others. Genotoxicity of pesticides varies from its complete absence (propiconazol as an example) to a very pronounced one	

		(captafol) with remaining compounds in between. These two compounds demonstrate full correlation between genotoxicity and carcinogenicity (or their absence). Many pesticides give positive results in some tests for genotoxicity but these results are frequently controversial, not readily reproducible, or obtained only at toxic dose levels. The weak genotoxicity of the majority of pesticides is easily explainable by their rather severe testing before their introduction into practical use. The above mechanisms are threshold-based and therefore pesticides are regulated through NOEL/safety factor. There exist examples of lack of correlation between genotoxicity and carcinogenicity: some pesticides are genotoxic (although not strongly) but noncarcinogenic, others are considered as nongenotoxic but are strongly carcinogenic (chlorothalonil, acetochlor). The general scheme of the promoters' effect is presented in which an important role is attributed to the cytochrome P-450 induction (some pesticides are the cytochrome P-450 inducers), formation of reactive oxygen species and peroxitome proliferation. Teratogenesis Carcinog. Mutagen. 20:229-240, 2000. Copyright 2000 Wiley-Liss, Inc. PMID: 10910473 [PubMed - indexed for MEDLINE] Related citations 5 Mutat Res. 1997 Oct 24;393(3):263-81. . **EVALUATION OF THE MUTAGENICITY OF ACETOCHLOR TO MALE RAT GERM CELLS.** Ashby J[1], Tinwell H, Lefevre PA, Williams J, Kier L, Adler ID, Clapp MJ. Author information: [1]Zeneca Central Toxicology Laboratory, Alderley Park, Cheshire,	

		UK. **Abstract** Male rat dominant lethal (DL) assays conducted on the herbicide acetochlor are described. Single dose studies conducted at the maximum tolerated dose (MTD, < or = 1000 mg/kg) produced no effects on any of the DL assay parameters at any of the ten weekly sampling periods. It is concluded that acetochlor is non-mutagenic to rat germ cells. Due to initial limited knowledge of the MTD of acetochlor it was also evaluated in the DL assay at a dose level of 2000 mg/kg. At this high dose level severe bodyweight loss and some deaths occurred among the treated animals. In addition, reduced implantations and reduced pregnancy rates were observed at the third sampling period (18-25 days post dosing) in the absence of an increase in early post-implantation deaths. These results indicated that the use of supra-MTD doses of acetochlor had reduced the fertility of the treated males leading to the production of a pseudo-DL assay response, as alerted to and defined by Ehling. Although several such pseudo-DL assay responses have been described, none have been explained mechanistically. It was therefore decided to pursue the effects seen in the DL assay when using supra-MTD doses of acetochlor. Ova analysis of female rats mated with male rats exposed to 2000 mg/kg acetochlor revealed unfertilized ova at the critical third sampling time. Normal fertilization of ova was observed at the first and fifth sampling period and, for a dose of 200 mg/kg acetochlor, at the third sampling period. The magnitude and temporal nature of these effects confirmed the induction of a pseudo-DL assay response, and studies were then undertaken to probe its genesis. Rats treated with 2000 mg/kg acetochlor had normal testicular and epididymal pathology and normal sperm numbers and sperm motility at the critical third sampling period. Despite a small reduction in	

		testicular and epididymal glutathione levels 12 h after exposure to 2000 mg/kg acetochlor, testicular LDH and LDH-X enzyme levels were unaffected. Further, no reduction in the level of free sulphydryl groups (-SH) were observed in epididymal caput sperm heads isolated 0.5, 7 or 14 days after treatment of male rats with 2000 mg/kg acetochlor. The only sperm parameter affected by treatment with 2000 mg/kg acetochlor was an increase in epididymal cauda sperm with head abnormalities. The non-specific nature of this effect was considered inadequate to explain fully the high dose fertility effects seen in the DL assays, which therefore remain unexplained. The present data establish that acetochlor is non-mutagenic to rat germ cells. They also confirm the importance of segregating mutagenic and fertility effects in the DL assay, and emphasize the need for appropriate dose-setting studies prior to the conduct of rodent genetic toxicity assays. PMID: 9393619 [PubMed - indexed for MEDLINE] Related citations	