

Micronucleus test and comet assay on mice fed over five generations a diet containing genetically modified triticale*

**K. Jaszczak^{1,4}, M. Kruszewski², A. Baranowski¹, R. Parada¹,
T. Bartłomiejczyk², J. Zimny³ and S. Rosochacki¹**

¹*Institute of Genetics and Animal Breeding,
Polish Academy of Sciences, Department of Molecular Cytogenetics
Jastrzębiec, 05-552 Wólka Kosowska, Poland*

²*Institute of Nuclear Chemistry and Technology,
Department of Radiobiology and Health Protection
Dorodna 16, 03-195 Warszawa, Poland*

³*Plant Breeding and Acclimatization Institute,
Department of Plant Biotechnology and Cytogenetics
Radzików, 05-870 Błonie, Poland*

(Received 25 May 2007; revised version 16 November 2007; accepted 15 January 2008)

ABSTRACT

One of the concerns regarding the common use of genetically modified (GM) feed in animal nutrition is that transgenic sequences may have a negative effect on the organism or (and) its cells. The present report assesses the genotoxic potential effect of a diet containing GM triticale on mice by using micronuclei test and comet assay. One group of mice (C57 Bl/6J strain) were fed continuously over five generations a pelleted diet containing 20% of GM triticale (tolerant to phosphinothricine) grain, while the control group was fed pellets with 20% conventional triticale grain. Ten 91-days-old mice (five females and five males) were randomly selected from each group and each generation for micronuclei test in bone marrow and peripheral blood erythrocytes and the some number of mice was used for comet assay. The results obtained did not reveal any statistically significant differences in the micronuclei frequency nor any other DNA damage between the control and experimental groups of mice in all five generations. Thus, it seems evident that the diet containing GM triticale (with *bar* transgene) does not induce chromosome damage, nor has it any effect on the formation of DNA breaks or base lesions.

KEY WORDS: GM triticale, multigeneration feeding, erythrocytes, micronucleus test, comet assay, mice

* Supported by the State Committee for Scientific Research, Grant No. 2 P06Z 055 26

⁴ Corresponding author: e-mail: K.Jaszczak@ighz.pl

INTRODUCTION

The use of new techniques to modify the genetic makeup of plants has led to a new generation of crops, grains and their by-products for feed (Flachowsky et al., 2005). One of the concerns regarding the use of GM food in animal nutrition is that the introduction of foreign DNA or the producing proteins in genetically modified plants may have an undesirable effect on the organism. However there is no direct evidence that GM food may pose a danger to the health and production of animals (Hammond et al., 1996; Donkin et al., 2000; Russell et al., 2001; Kosieradzka et al., 2004; Baranowski et al., 2006). Some published reports suggest that consumption of GM feed by animals may lead to negative changes in their organisms. Ultrastructural morphometrical and immunocytochemical analyses on hepatocytes nuclei from mice fed GM soyabean demonstrate significant modifications of some nuclear features in GM-fed mice. In particular GM-fed mice show an irregularly shaped nuclei, which generally represent an index of high metabolic rate (Malatesta et al., 2002). Diets containing GM potatoes had variable effects on different parts of the rats' gastrointestinal tract. Some effects, such as the proliferation of the gastric mucosa, were due principally to the expression of the GNA transgene (Ewen and Pusztai, 1999). It is a known fact that it is possible to transfer transgene fragments from GM plants to intestine microflora, enterocytes and other tissues of animals and human (Einspanier et al., 2001; Phipps et al., 2003; Netherwood et al., 2004; Mazza et al., 2005; Sharma et al., 2006).

Foreign DNA, orally ingested by pregnant mice, was discovered in various organs of foetuses and of newborn animals (Schubbert et al., 1998). Authors considered that maternally ingested foreign DNA could be a potential mutagen for the developing foetuses. Inserting foreign DNA into mammalian genome can lead to alterations in cellular DNA methylation and transcription patterns (Muller et al., 2001).

The *in vivo* micronucleus assay in mammalian bone marrow and peripheral blood erythrocytes is a rapid screen for the detection of chromosomal damage caused by a genotoxic agents (Schmid, 1975).

Single cell gel electrophoresis (SCGE), also known as comet assay, is a sensitive method for measuring DNA strand breaks in animal and plant cells (McKelvey-Martin et al., 1993; Tice et al., 2000).

The reports cited have led to some questions about the safety of GM plants used as feed for animals and made it necessary to undertake complex investigations of any new GM variety and in particular when it is a feeding plant.

The work described in the present paper aimed to investigate the possible genotoxic effect of a diet containing a GM plant by micronucleus test in bone marrow and peripheral blood cells and comet assay in mice continuously fed for several generations GM triticale.

MATERIAL AND METHODS

Animals

All experiments were conducted on the C57BL/6J strain of mice. One group of animals for 5 generations received pellets containing 20% of GM triticale grain (transgenic diet), while the second group was fed pellets containing 20% of a conventional triticale grain (control diet). Pelleted diet for control group and another one for experimental group were both prepared in the feed-producing plant specialized in feeds for laboratory rodents (certified by Polish Standard PN ISO 9001). Pellets for both groups (Table 1) contained neither additives of

Table 1. Composition of conventional (control) and experimental diet, %

Component	Control diet	Experimental diet
Maize grain	20.0	20.0
Conventional triticale	20.0	-
Transgenic triticale	-	20.0
Oat grain (defatted)	10.0	10.0
Soyabean oilmeal	27.1	27.1
Wheat bran	15.0	15.0
Yeast	4.0	4.0
Ca-phosphate	1.0	1.0
Chalk	1.5	1.5
NaCl	0.4	0.4
Premix LRM, DSM Nutritional Products, Poland	1.0	1.0

animal origin, pharmaceuticals, and probiotics nor GM products (other than transgenic triticale in pellets for experimental group), and were completely balanced as far as protein, energy and mineral-vitamin additives are concerned. From a pool of conventional and transgenic triticale grain as well as from respective pellets 1 kg samples were withdrawn (n=4) to perform proximate analyses as well as to confirm the presence of transgene by PCR (Table 2).

Table 2. Proximate composition of control and experimental diet

Item	Control diet n=4	Experimental diet n=4
Dry matter, %	87.45 ± 1.76	86.94 ± 0.52
In dry matter, %		
protein, N × 6.25	25.15 ± 0.31	25.00 ± 0.18
crude fibre	4.91 ± 1.11	4.77 ± 1.34
ether extract	3.13 ± 0.41	3.10 ± 0.58
ash	7.08 ± 0.40	7.47 ± 0.44
N-free extractives	59.73 ± 0.97	59.66 ± 1.41

The transgenic triticale (hybrid of *Triticum durum* and *Secale cereale*) was obtained as described by Zimny et al. (1995). Briefly, triticale explants were bombarded with gold particles coated with plasmid DNA. The plasmid used (pDB1) contained, in particular, the bacterial gene *bar* as a selectable marker. The expression and integration of the bacterial gene were confirmed by biological (tolerance of phosphinothricine) and biochemical (PCR) tests.

Ten 91-days-old mice (five females and five males) were selected randomly from the offspring of 15 parental pairs, in each group and generation, for micronucleus test in bone marrow and blood cells and the same number of mice for comet assay.

During the experiment the animals were maintained at the our mice farm where all the regulations of rodent keeping and breeding have been observed. The mice were housed in plastic cages with wire tops, and were supplied *ad libitum* with their respective control or experimental pellets, with free access to water. Detail conditions of the maintenance and breeding of mice during the experiment were described in a previous report by Baranowski et al. (2006). This experiment was approved by the Local Commission for Ethics in Animal Experiments.

Micronucleus test

The preparation and staining of bone marrow cells for micronucleus test were carried out according to a standardized procedure based on that of Schmid (1975) and Mavournin et al. (1990). The mice were killed by cervical dislocation. The bone marrow cells were flushed out from the femur with foetal calf serum (Sigma-Aldrich, St Louis, MO, USA), centrifuged, smeared and stained with May-Grünwald and Giemsa solution (Sigma-Aldrich, St Louis, MO, USA). To determine the frequency of micronuclei in bone marrow cells an examination was made of 1000 polychromatic erythrocytes from each animal.

Blood smear slides were prepared by the same method as that used for bone marrow samples. Micronuclei in peripheral blood were scored in 1000 normochromatic and polychromatic erythrocytes from each animal. All slides were coded and scored blind.

Comet assay

DNA damage was estimated using alkaline comet assay according to the following protocol. This version of the comet assay is used to estimate DNA strand alternation, such as single strand breaks (ssb), double strand breaks and alkali labile sites. The comet assay, modified by use of DNA glycosylase (FPG), was used to estimate the oxidation of DNA bases (FPG sensitive sites). The primary substrate for FPG was 7,8-dihydro-8-oxoguanine (8oxoG), one of the most frequently occurring base modification in human DNA (Frosina, 2006).

Single strand DNA breaks were estimated according to the standard protocol described in detail by Wojewódzka et al. (1998). 8-oxoG was estimated according to the protocol described by Kruszewski et al. (1998).

In brief, microscope slides were precoated with 100 μ l of agarose (0.5% Agarose Type I-A (Sigma-Aldrich, St Louis, MO, USA), in redistilled water and dried. Thirty microliters of whole blood were added to 1 ml of RPMI 1640 medium (Sigma-Aldrich, St Louis, MO, USA) containing 10% FCS (Sigma-Aldrich, St Louis, MO, USA) and incubated for 2.5 h (in darkness at room temperature). One hundred microliters of cell suspension in 1% LMP agarose (Type VII, Sigma-Aldrich, St Louis, MO, USA) in PBS were casted on precoated slides and allowed to set on ice. About $2\text{-}5 \times 10^4$ cells were casted per slide. Once agarose set, slides were lised in 2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 (Sigma-Aldrich, St Louis, MO, USA), pH=10 and left overnight at 4°C in dark. Slides for FPG treatment were washed 3×5 min with Hepes buffer (40 mM Hepes, 0.1 M KCl, 0.5 mM EDTA, 0.2 mg/ml BSA), at 4°C. Then, 50 μ l of FPG enzyme (New England BioLabs, UK) diluted 1:10000, were added dropwise on the agarose, and incubated for 30 min at 37°C in humidity. During this manipulation, slides for ssb estimation were kept in lysis buffer. Slides were then placed in electrophoresis apparatus (Bio-Rad Laboratories, Hercules, California) in a electrophoresis buffer (300 mM NaOH, 1 mM EDTA (Sigma-Aldrich, St Louis, MO, USA) and left for 40 min at 10°C, for unwinding. Electrophoresis was carried out for 30 min at 1.2 V/cm. All steps of slide preparation and electrophoresis were conducted at the red light or in dark to avoid induction of additional DNA damage. After electrophoresis, slides were washed three times in neutralizing buffer 0.4 M Tris (Sigma-Aldrich, St Louis, MO, USA), pH=7.5, dried from the excess of buffer and stained 1 μ M DAPI (Sigma-Aldrich, St Louis, MO, USA). Slides were left at 4°C in humidity overnight and scored.

Comets were scored with computer aided image analysis system Comet v.3.0 (Kinetic Imaging Ltd., Liverpool, UK). Tail moment was taken as a measure of DNA damage.

Statistical analysis

The results were subjected to variance analysis using the least squares method (Harvey, 1990). The following model was used:

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$$

where: Y_{ijk} - the analysed trait, μ - overall means, a_i - effect of diet ($i = 1$ -transgenic, 2 - control), b_j - effect of generation ($j = 1\text{...}5$), $(ab)_{ij}$ - interaction (diet \times generation), e_{ijk} - error.

RESULTS AND DISCUSSION

The results of the micronucleus test in bone marrow and peripheral blood erythrocytes of mice fed conventional or GM triticale for five generations are shown in Table 3. The frequency of micronuclei in bone marrow ranged from

Table 3. Frequency (% \pm SD) of micronuclei in bone marrow and blood erythrocytes of mice fed a transgenic or control diet over 5 generations

Diet	Generation	Number of animals	Bone marrow	Erythrocytes
			LSM \pm SD	LSM \pm SD
GM triticale	F1	10	0.69 \pm 0.20	0.43 \pm 0.26
		10	0.56 \pm 0.19	0.61 \pm 0.46
Control	F1		NS	NS
GM triticale	F2	10	0.51 \pm 0.11	0.52 \pm 0.10
		10	0.48 \pm 0.09	0.45 \pm 0.09
Control	F2		NS	NS
GM triticale	F3	10	0.50 \pm 0.08	0.57 \pm 0.11
		10	0.47 \pm 0.09	0.47 \pm 0.09
Control	F3		NS	NS
GM triticale	F4	10	0.54 \pm 0.11	0.65 \pm 0.34
		10	0.47 \pm 0.06	0.44 \pm 0.08
Control	F4		NS	NS
GM triticale	F5	10	0.68 \pm 0.19	0.37 \pm 0.14
		10	0.68 \pm 0.22	0.59 \pm 0.33
Control	F5		NS	NS

NS - not significant

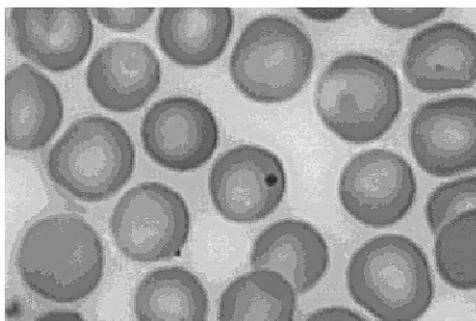


Figure 1. Peripheral blood smear, the arrows point at the micronucleus in the erythrocyte

0.50 to 0.69% for experimental groups (fed GM triticale) and from 0.47 to 0.68% for control groups (fed conventional triticale). In the peripheral blood the frequency of micronuclei (Figure 1) ranged from 0.37 to 0.65% for experimental groups and from 0.44 to 0.61% for control groups. The micronuclei frequency in bone marrow and blood erythrocytes showed no significant changes over five

generations and their level was similar to spontaneous values given for different mice strains (Bhilwade et al., 2004).

The micronucleus test of mice erythrocytes is especially useful for the evaluation of the threat of mutagenic activity, since it enables to regard the metabolic factors and DNA repair processes *in vivo*, and those can differ depending on species, strain and tissue (Hamada et al., 2001; Bhilwade et al., 2004).

The *in vivo* appearance of micronuclei is predominantly a result of chromosome loss, a non abundant DNA lesion that leads to aneuploidy and cell death (Tweats et al., 2007).

To investigate the effect of GM triticale on more frequent DNA lesions, such as DNA single strand breaks and base damage, the comet assay was used (Figure 2). Both DNA single strand breaks and base damage are common DNA lesions,

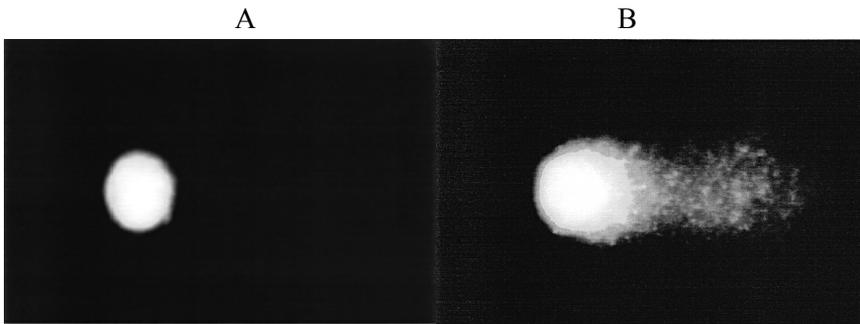


Figure 2. Comet assay: image of normal (A) and damaged nuclei cell (B)

formed during normal cellular aerobic metabolism (Cooke et al., 2003). The results of the comet assay of mice fed conventional or GM triticale for five generations are shown in Table 4. No statistically significant difference was observed between control and experimental groups, neither in case of DNA single strand breaks nor FPG sensitive sites in all five generations. Although comet assay is very well suited to the *in vivo* DNA damage investigation (Burlinson et al., 2007), a considerable differences were observed between different donor animals in course of this study (Table 2). Similar high diversity in DNA damage has been reported previously between human blood donors (Grzesiuk et al., 2006) and can be explained by differences in everyday individual behaviour of blood donors (for review, see Møller, 2000).

The diet with transgenic triticale did not affect in this experiment the postnatal growth and development of mice and polymerase chain reaction used to analyses several tissues did not show the presence of transgenic DNA (PCR product of 637 bp) in any of the examined tissues: blood, kidney, liver, spleen and thigh

Table 4. Frequency (% \pm SD) of DNA breaks and alkali labile sites in mice fed a transgenic or control diet over 5 generations

Diet	Generation	Number of animals	DNA breaks	Number of animals	Alkali labile sites
			LSM \pm SD		LSM \pm SD
GM triticale Control	F1	8	21.28 \pm 28.41	8	70.24 \pm 34.92
		8	16.46 \pm 6.57	8	127.50 \pm 44.05
			NS		NS
GM triticale Control	F2	4	25.88 \pm 4.97	-	-
		6	37.45 \pm 16.91	-	-
			NS		
GM triticale Control	F3	7	30.58 \pm 20.81	7	84.64 \pm 33.36
		7	19.23 \pm 4.63	7	103.63 \pm 26.36
			NS		NS
GM triticale Control	F4	8	31.79 \pm 22.04	8	93.88 \pm 44.55
		8	24.20 \pm 10.20	8	75.89 ^x \pm 17.21
			NS		0.024
GM triticale Control	F5	8	21.10 \pm 12.96	7	40.91 \pm 15.03
		8	10.29 \pm 4.32	6	51.45 \pm 36.96
			NS		NS

NS - not significant

X - interaction (generation \times diet)

muscle (Baranowski et al., 2006). Similar results were obtained in multigeneration reproductive and developmental toxicity study of *bar* gene inserted into genetically modified potato on rats (Rhee et al., 2005).

In the literature available there is no data concerning genotoxic activity of diet-derived DNA on animal cells after consumption of GM plants.

CONCLUSIONS

The results obtained in our experiment show clearly that the diet containing GM triticale with *bar* gene tolerant to phosphinothricine, as an active substance of herbicide BASTA, does not induce chromosome damages, nor has it any effect on the formation of DNA breaks or base lesions.

ACKNOWLEDGEMENTS

The authors express their gratitude to Professor J. Klewiec for statistical evaluation of results.

REFERENCES

- Baranowski A., Rosochacki S.J., Parada R., Jaszczak K., Zimny J., Połozynowicz J., 2006. The effect of diet containing genetically modified triticale on growth and transgenic DNA fate in selected tissues of mice. *Anim. Sci. Pap. Rep.* 24, 129-142
- Bhilwade H.N., Chaubey R.C., Chauhan P.S., 2004. Gamma ray induced bone marrow micronucleated erythrocytes in seven strains of mouse. *Mutat. Res.* 560, 19-26
- Burlinson B., Tice R.R., Speit G., Agurell E., Brendler-Schwaab S.Y., Collins A.R., Escobar P., Honma M., Kumaravel T.S., Nakajima M., Sasaki Y.F., Thybaud V., Uno Y., Vasquez M., Hartmann A., 2007. Fourth International Workgroup on Genotoxicity Testing: Results of the in vivo Comet assay workgroup. *Mutat. Res.* 627, 31-35
- Cooke M.S., Evans M.D., Dizdaroglu M., Lunec J., 2003. Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J.* 17, 1195-1214
- Donkin S.S., Velez J.C., Totten A.K., Stanisiewski E.P., Hartnell G.F., 2000. Effect of feeding Roundup Ready corn silage and grain on feed intake, milk production and milk composition in lactating dairy cattle. *J. Dairy Sci.* 83, Suppl. 1, 273 (Abstr.)
- Einspanier R., Klotz A., Kraft J., Aulrich K., Poser R., Schwägele F., Jahreis G., Flachowsky G., 2001. The fate of forage plant DNA in farm animals: A collaborative case-study investigating cattle and chicken fed recombinant plant material. *Eur. Food Res. Technol.* 212, 129-134
- Ewen S.W., Pusztai A., 1999. Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. *Lancet* 16, 354, 1353-1354
- Flachowsky G., Chesson A., Aulrich K., 2005. Animal nutrition with feeds from genetically modified plants. *Arch. Anim. Nutr.* 59, 1-40
- Frosina G., 2006. Prophylaxis of oxidative DNA damage by formamidopyrimidine-DNA glycosylase. *Int. J. Cancer* 119, 1-7
- Grzesiuk W., Nieminuszczy J., Kruszewski M., Iwanienko T., Plazinska M., Bogdanska M., Bar-Andziak E., Krolicki L., Grzesiuk E., 2006. DNA damage and its repair in lymphocytes and thyroid nodule cells during radioiodine therapy in patients with hyperthyroidism. *J. Mol. Endocrinol.* 37, 527-532
- Hamada S., Yamasaki K., Nakanishi S., Omori T., Serikawa T., Hayashi M., 2001. Evaluation of the general suitability of the rat for the micronucleus assay: the effect of cyclophosphamide in 14 strains. *Mutat. Res.* 495, 127-134
- Hammond B.G., Vicini J.L., Hartnell G.F., Naylor M.W., Knight C.D., Robinson E.H., Fuchs R.L., Padgett S.R., 1996. The feeding value of soybeans fed to rats, chickens, catfish and dairy cattle is not altered by genetic incorporation of glyphosate tolerance. *J. Nutr.* 126, 717-727
- Harvey W.R., 1990. Mixed Model Least-Squares and Maximum Likelihood Computer Program. PC-2 Version, 1-92. Ohio State University (USA)
- Kosieradzka I., Sawosz E., Pastuszewska B., Żuk M., Szopa J., Bielecki W., 2004. Effect of feeding potato tubers modified by 14-3-3 protein overexpression on metabolism and health status of rats. *J. Anim. Feed Sci.* 13, 329-339
- Kruszewski M., Wojewódzka M., Iwaneńko T., Collins A.R., Szumiel I., 1998. Application of the comet assay for monitoring DNA damage in workers exposed to chronic low dose irradiation. II. Base damage. *Mutat. Res.* 416, 37-57
- Malatesta M., Caporaloni C., Gavaudan S., Rocchi M.B., Serafini S., Tiberi C., Gazzanelli G., 2002. Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. *Cell Struct. Funct.* 27, 173-180. Erratum in: *Cell Struct. Funct.* 27, 399

- Mavournin K.H., Blakey D.H., Cimino M.C., Salamone M.F., Heddle J.A., 1990. The *in vivo* micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* 239, 29-80
- Mazza R., Soave M., Morlacchini M., Piva G., Marocco A., 2005. Assessing the transfer of genetically modified DNA from feed to animal tissues. *Transgenic Res.* 14, 775-784
- McKelvey-Martin V.J., Green M.H., Schmezer P., Pool-Zobel B.L., De Meo M.P., Collins A., 1993. The single cell gel electrophoresis assay (comet assay): a European review. *Mutat. Res.* 288, 47-63
- Møller P., Knudsen L.E., Loft S., Wallin H., 2000. The Comet Assay as a Rapid Test in Biomonitoring Occupational Exposure to DNA-damaging Agents and Effect of Confounding Factors. *Cancer Epidemiology Biomarkers & Prevention*, Vol. 9, pp. 1005-1015
- Muller K., Heller H., Doerfler W., 2001. Foreign DNA integration. Genome-wide perturbations of methylation and transcription in the recipient genomes. *J. Biol. Chem.* 276, 14271-14278
- Netherwood T., Martin-Orue S.M., O'Donnell A.G., Gockling S., Graham J., Mathers J.C., Gilbert H.J., 2004. Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. *Nat. Biotechnol.* 22, 204-209
- Phipps R.H., Deaville E.R., Maddison B.C., 2003. Detection of transgenic and endogenous plant DNA in rumen fluid, duodenal digesta, milk, blood, and feces of lactating dairy cows. *J. Dairy Sci.* 86, 4070-4078
- Rhee G.S., Cho D.H., Won Y.H., Seok J.H., Kim S.S., Kwack S.J., Lee R.D., Chae S.Y., Kim J.W., Lee B.M., Park K.L., Choi K.S., 2005. Multigeneration reproductive and developmental toxicity study of bar gene inserted into genetically modified potato on rats. *J. Toxicol. Environ. Health Pt A*, 68, 2263-2276
- Russell J.R., Hersom M.J., Haan M.M., Kruse M.L., Morrill D.G., 2001. Effects of grazing crop residues from Bt-corn hybrids on pregnant beef cows. *J. Anim. Sci.* 79, Suppl. 2, 98 (Abstr. 300)
- Schmid W., 1975. The micronucleus test. *Mutat. Res.* 31, 9-15
- Schubert R., Hohlweg U., Renz D., Doerfler W., 1998. On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. *Mol. Gen. Genet.* 259, 569-576
- Sharma R., Damgaard D., Alexander T.W., Dugan M.E., Aalhus J.L., Stanford K., McAllister T.A., 2006. Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal. *J. Agr. Food Chem.* 54, 1699-1709
- Tice R.R., Agurell E., Anderson D., Burlinson B., Hartmann A., Kobayashi H., Miyamae Y., Rojas E., Ryu J.C., Sasaki Y.F., 2000. Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. *Environ. Mol. Mutagen.* 35, 206-221
- Tweats D.J., Blakey D., Heflich R.H., Jacobs A., Jacobsen S.D., Morita T., Nohmi T., O'Donovan M.R., Sasaki T., Sofuni R., Tice R.R., 2007. Report of the IWGT working group on strategy/interpretation for regulatory *in vivo* tests: II. Identification of *in vivo*-only positive compounds in the bone marrow micronucleus test. *Mutat. Res.* 627, 92-105
- Wojewódzka M., Kruszewski M., Iwaneńko T., Collins A.R., Szumiel I., 1998. Application of the comet assay for monitoring DNA damage in workers exposed to chronic low dose irradiation. I. Total damage. *Mutat. Res.* 416, 21-35
- Zimny J., Becker D., Brettschneider R., Lörz H., 1995. Fertile transgenic *triticale* (*x Triticosecale* Wittmack). *Mol. Breeding* 1, 155-164