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Heritable translocations induced by dermal exposure of male mice to acrylamide

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Abstract. Acrylamide (AA) is an important industrial chemical used mainly in the production of polymers. It can be absorbed through the skin. AA was shown to be a germ cell clastogen that entails a genetic risk for exposed workers. The genetic risk calculation was based on mouse heritable translocation test data obtained after acute intraperitoneal (ip) exposure (Adler et al., 1994). To obtain a correction factor between ip and dermal exposure, dominant lethal and heritable translocation tests were carried out with dermal exposure of male mice to AA. In the dominant lethal test, male (102/El × C3H/El)F_1 mice were exposed by dermal application to the shaved backs of 50 mg/kg AA per day on five consecutive days or to five daily ip injections of 50 mg/kg AA. One day after the end of exposure, the males were mated to untreated females of the same hybrid stock for four days and females were changed every four days for a total of five matings. Dominant lethal effects were found during matings 1–3. For ip exposure, these values were 81.7, 85.7 and 45.4%, respectively; for dermal exposure the corresponding values were 22.1, 30.6 and 16.5%, respectively. In the heritable translocation assay, male C3H/El mice were treated with five dermal exposures of 50 mg/kg AA and mated 1.5–8.5 days after the end of exposure to untreated female 102/El mice. Pregnant females were allowed to come to term and all offspring were raised to maturity. Translocation carriers among the F_1 progeny were selected by a sequential fertility testing and cytogenetic analysis including G-band karyotyping and M-FISH. A total of 475 offspring were screened and 41 translocation carriers were identified. The observed translocation frequency after dermal exposure was 8.6% as compared to 21.9% after similar ip exposure (Adler, 1990). The calculated ratio of ip vs. dermal exposure of 0.39 can be applied to obtain a more realistic calculation of genetic risk for dermally exposed workers.

Acrylamide (AA) is a monomeric chemical widely used in the production of AA polymer flocculants, polyacrylamide gels for electrophoresis, thickening agent, filtration aid and grouting agent. A Swedish group demonstrated that AA is formed during heating of starch-rich foods to high temperatures (Tareke et al., 2000, 2002), which raised a new wave of human health concern.

The neurotoxicity of AA was established quite early and led to the regulation of AA (Tilson, 1981; Miller and Spencer, 1985; O'Donoghue, 1985). Recently, a review on the effects of AA on rodent reproduction was published (Tyl and Friedman, 2003). The evidence that AA was carcinogenic in rodents (Bull et al., 1984; Johnson et al., 1986) increased the concern for AA as a potential human health hazard. Further investigations of AA identified its genotoxicity (Dearfield et al., 1988). Interestingly, AA was not mutagenic in the Ames test (Lijinsky and Andrews, 1980; Bull et al., 1984; Hashimoto and Tanii, 1985). It appeared that the major genotoxicity effect of AA was its clastogenic activity (Shiraishi, 1978; Moore et al., 1987; Adler et al., 1988; Knaap et al., 1988). Using flow cytometer-based analyses, it was demonstrated that the dose-response relationship for AA-induced micronuclei in mouse bone marrow cells was linear down to low single doses of 1–30 mg/kg AA applied by intraperitoneal (ip) injection (Abramsson-Zetterberg, 2003). In rodent germ cells, dominant lethal mutations and heritable translocations were induced by ip injection with AA in late spermatids and sperm (Adler, 1990; Shelby et al., 1986, 1987; Smith et al., 1986). Analysis of structural chromosome aberra-

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tions in one-cell embryos after paternal AA-treatment was performed using conventional and molecular techniques (Pacchierotti et al., 1994; Marchetti et al., 1997). The data obtained allowed an estimate of the magnitude of AA-induced dominant lethality and heritable translocations showing close correlation of the results obtained by the respective tests. Even though the methodology of one-cell embryo collection and cytogenetic analysis requires great skills and is time-consuming, it may offer an alternative to the animal-consuming dominant lethal and heritable translocation assays. Sperm-chromatin alkylation was measured by accelerator mass spectrometry after ip treatment of male mice with ^{14}C-acrylamide (Holland et al., 1999). It was correlated to pre-implantation losses of embryos in concurrent analyses of embryonic development in vitro and pre- and postimplantation loss of embryos in a dominant lethal assay with AA. The study confirmed the stage sensitivity of spermatogenesis to AA observed in the earlier dominant lethal studies. While specific locus mutations were observed originally in the same germ cell stages as the dominant lethal mutations (Russell et al., 1991), it was shown later that AA also induced mutations in spermatogonial stem cells of mice (Ehling and Neuhäuser-Klaus, 1992). Originally, alkylation of chromosomal protamines in late spermatids and sperm was assumed to cause the clastogenic effects in male germ cells (Sega et al., 1989). However, it could be demonstrated later that glycidamide was the genotoxic metabolite of AA in somatic and germinal cells of rodents (Adler et al., 2000; Generoso et al., 1996; Paulsson et al., 2003).

The main human exposure at production sites, in biochemical laboratories or during accidents in tunnel construction, where AA was used for gap filling in the rocks, occurred via dermal contact and to a lesser extent by ingestion. In mice, AA is readily absorbed through the skin and binds to DNA in the testes (Carlson and Weaver, 1985). It could be shown that dermal exposure of mice induced a dose-dependent increase of dominant lethal mutations in male mice (Gutierrez-Espeleta et al., 1992).

A dose response for the induction of heritable translocations in mouse spermatids by ip exposure to AA and a calculation of the possible genetic risk from AA-exposure of humans was published earlier (Adler et al., 1994). However, it seemed necessary to determine a conversion factor from ip to dermal exposure to derive a more realistic genetic risk calculation for exposed workers. The present dominant lethal and heritable translocation experiments were performed to compare the clastogenic effects of AA in mouse male germ cells on a quantitative basis between ip and dermal exposure.

Materials and methods

Chemical and dosing

Acrylamide (AA) was obtained from Sigma, Deisenhofen, Germany. In the dominant lethal assay, male mice were treated with five daily ip injections or five daily dermal applications on the shaved backs with 50 mg/kg of AA dissolved in saline (ip) or corn oil (dermal). The applied volume was 0.1 ml/10 g body weight. Control animals received the same application of the solvents. The heritable translocation assay was performed with dermal exposure only.

Animals

All animals were bred and maintained in the animal facility of the GSF-Research Center in light- and air-conditioned animal rooms (12L/12D, 25 °C, 55 % humidity) and received pellet food and water ad libitum.

For the dominant lethal study, (102/El × C3H/El)F_1 males, age 12–14 weeks, weighing between 24 and 26 g, were treated and mated to untreated virgin females of the same age and stock at a 1:1 ratio, starting one day after the end of treatment. Females were replaced four times every four days for a total of five mating intervals. On days 13–14 post conception (pc), the uterus contents of the females were inspected for live and dead implants. Percent induced dominant lethal was calculated by the formula (1 – [live implants per female in the treated group/live implants per female in the control group]) × 100.

For the heritable translocation assay, males of the C3H/El inbred strain, age 10–12 weeks, weighing between 24 and 26 g, were treated by dermal application of 50 mg/kg of AA on five consecutive days and mated 1.5–8.5 days after the end of treatment to untreated virgin 102/El females of the same age at a mating ratio of 1:2. The experiment was repeated once. Pregnant females were allowed to come to term. Litters were counted and sexed at birth and weaned at the age of 3 weeks.

Selection of translocation-suspect F_1 animals by litter size reduction

F_1 progeny of both sexes were mated at the age of 10–12 weeks avoiding brother-sister matings. To determine possible translocation heterozygotes by reduced fertility a sequential decision procedure of eliminating pairs with normal litters was employed (Adler, 1990). Up to three litters were observed before F_1 pairs with reduced litter size or pairs without any litter were separated.

Confirmation of translocation-suspect F_1 males

Suspect F_1 males were mated to 4–5 (102/El × C3H/El) F_1 females. Females were sacrificed at mid-pregnancy to determine the frequency of early dead implants. Males that impregnated females with an average of three or more dead implants were subjected to cytogenetic confirmation of the translocation in testis preparations (Evans et al., 1964). F_1 males identified as translocation heterozygotes were subjected to karyotype analysis in Giemsa-banded bone marrow preparations (Gallimore and Richardson, 1973; Evans, 1989; Adler et al., 2002) or by M-FISH (Jentsch et al., 2001).

Confirmation of translocation-suspect F_1 females

Suspect F_1 females were mated to (102/El × C3H/El) F_1 males and were again allowed to have up to four litters. Male F_2 progeny from small litters of suspect F_1 females were weaned and subjected to meiotic chromosome analysis at maturity as described above. Translocation-heterozygous females were confirmed by the presence of at least one translocation carrier among eight F_2 males. The F_1 translocation females or their translocation-carrying offspring were subjected to karyotype analysis of Giemsa-banded bone marrow chromosomes or by M-FISH as stated above.

Statistics

Dominant lethal results were compared between treatment and solvent control groups on a male-to-male basis using the Mann-Whitney U test. Differences in translocation frequencies between the experimental group and the historical control group were analysed using Fisher's exact test (one-sided).

Results and discussion

Table 1 shows the results of the dominant lethal experiments. The frequencies of dead implants per female are increased with both modes of exposure during the first three mating intervals, which represent sperm and late spermatids. This pattern of sensitivity is in accord with previous publications (Shelby et al., 1986, 1987; Smith et al., 1986, Holland et al., 1999). The dominant lethal effects are illustrated in Fig. 1. The effect was 2–4 times higher after ip exposure compared to dermal exposure. The results after dermal exposure are compa-

rable to a previous publication (Gutierrez-Espeleta et al., 1992).

The results of the heritable translocation assay are shown in Table 2. In the first experiment, 242 F_1 progeny (138 males and 104 females) were tested for reduced fertility and 19 (12 males and 7 females) were identified to be translocation carriers. In the second experiment 233 F_1 progeny (120 males and 113 females) were tested for reduced fertility and 22 (16 males and 6 females) were identified to carry translocations. The observed translocation rates of the two experiments (7.85 and 9.44%, respectively) were not significantly different. Therefore, the data were pooled. The observed mean translocation frequency of 8.63% was significantly different from the historical control in our laboratory (Adler et al., 2002).

Table 3 shows the characteristics of the identified male translocation carriers from both experiments. Nine sterile F_1 translocation males were found. One of these carried a reciprocal translocation plus an inversion in chromosome 3 (AAD-229: T[9;13]+Inv.3, mean testis weight 60.9 mg), one carried two independent reciprocal translocations (AAD-240: T[1;12]

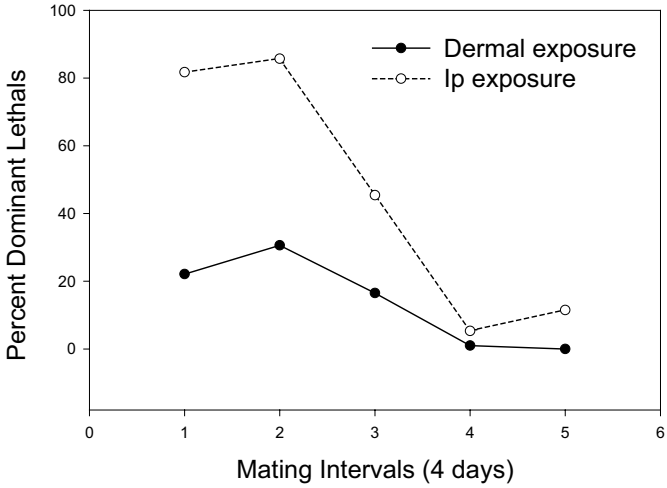

Fig. 1. Dominant lethal effects in sperm and spermatids of male mice after intraperitoneal (ip) and dermal exposure to 5 × 50 mg/kg of acrylamide. Percent dominant lethals = (1 – [live implants per female in the treated group/ live implants per female in the control group]) × 100.

Table 1. Results with the dominant lethal test after five daily exposures of male mice to 50 mg/kg of acrylamide

Mating intervals[a] (days)	Application	Pregnant females[b]		Total implants		Live implants (LI)		Dead implants (DI)		% DI	Dominant lethals[c]
		n	%	n	Per female	n	Per female	n	Per female		
1.5–4.5	dermal	45	90	470	10.4	363	8.1	107	2.38[d]	22.8	22.1
	ip	27	60	159	5.9	52	1.9	107	3.96[d]	76.3	81.7
	control	50	100	561	11.2	519	10.4	42	0.84	7.5	–
5.4–8.5	dermal	48	96	478	10.0	328	6.8	150	3.13[d]	31.4	30.6
	ip	35	78	191	5.5	49	1.4	142	4.06[d]	74.4	85.7
	control	48	96	514	10.7	468	9.8	46	0.96	9.0	–
9.5–12.5	dermal	43	86	454	10.6	350	8.1	104	2.42[d]	22.9	16.5
	ip	41	91	368	9.0	216	5.3	152	3.71[d]	41.3	45.4
	conrol	43	86	465	10.8	419	9.7	46	1.07	10.0	–
13.5–16.5	dermal	44	88	455	10.3	414	9.4	41	0.93	9.0	1.0
	ip	36	80	364	10.1	323	9.0	41	1.14	11.3	5.3
	control	40	80	417	10.4	380	9.5	37	0.93	8.9	–
17.5–20.5	dermal	38	76	434	11.4	397	10.5	37	0.97	8.5	0
	ip	41	91	430	10.5	383	9.3	47	1.15	10.9	11.5
	control	46	92	535	11.6	481	10.5	54	1.17	10.1	–

[a] Mating started in the afternoon of the day after the end of exposure.
[b] Mating ratio 1:1, 50 males treated per group, five males died in the ip group during the course of treatment.
[c] Dominant lethals (%) = (1 – [LI per female $_{exp.}$/LI per female $_{contr.}$]) × 100.
[d] $P < 0.05$.

Table 2. Results of the heritable translocation test after five daily dermal exposures of male mice to 50 mg/kg of acrylamide (two repeats)

Experiment	Males treated	Females mated	Litters	F_1 progeny tested	Translocation carriers	Translocation rate
1	50	100	63	242 (138♂ / 104♀)	19 (12♂ / 7♀)	7.85 %
2	50	100	73	233 (120♂ / 113♀)	22 (16♂ / 6♀)	9.44 %
Total	100	200	136	475 (258♂ / 117♀)	41 (28♂ / 13♀)	8.63 %[a]

[a] $P < 0.001$ compared to the historical control of 0.05%

Table 3. Characterization of the male translocation carriers

Animal code	Mean litter size[a]	Translocation multivalents[b] (%)	Chromosomes involved[c]	Presumed break points
First experiment				
AAD-5	5.7	9.3	T(14;19)	14E2.2; 19D1
AAD-34	2.8	40	T (2;7)	2H3; 7D1
AAD-37	st	96	T(5;19)	5EF; 19B
AAD-40	3.4	84	T(13;14)	13C3; 14A3
AAD-56	3.3	5.2	T(4;9)	4D3; 9A4
AAD-58	3.6	39	T(5;19)	5B2; 19B
AAD-67	3.2	88	T(9;1)	9B; 11B3
AAD-83	2.0	100 (44% 2TM)	nd	nd
AAD-86	st	no meioses	T(3;5)	3F1; 5F
AAD-89	3.5	28	T(9;16)	9B; 16B5
AAD-96	2.3	47	T(4;8)	4C3; 8B3.2
AAD-127	st	88	T(7;10)	7F1; 10A4
Second experiment				
AAD-155	4.0	13	T(2;15)	2G1; 15C
AAD-164	6.3	76	T(11;17)	11D; 17D
AAD-165	st	70	T(14;17;19)	14E2; 17E2; 19C2
AAD-169	3.8	20	T(9;13)	9E3; 13C2
AAD-181	st	6	T(9;Y)	9F1; YD
AAD-185	3.5	64	T(1;7)	1D; 7F1
AAD-198	5.5	36	T(4;8)	4C6; 8E1
AAD-215	st	no meioses	T(3;4)	3E2; 4D3
AAD-216	4.7	80	T(4;7)	nd
AAD-225	3.5	36	T(10;14)	10C1; 14B
AAD-227	st	20 (no sperm)	T(9;Y)	9F1; YC2
AAD-229	st	80	T(9;13)+Inv 3	9F1; 13D1; 3E2/G2
AAD-233	3.3	76	T(12;17)	12D2; 17B1
AAD-236	3.8	56	T(4;8)	4B3; 8D3
AAD-240	st	no meioses	T(1;12)+T(5;10)	1F; 12C3; 5A2; 10D2
AAD-253	3.3	56	T(3;14)	3H3; 14B

[a] From 3–5 litters, st = sterile.
[b] Per animal, 25 spermatocytes at diakineses were scored for translocation multivalents (chains of four or rings of four chromosomes).
[c] nd = not determined.

Table 4. Characterization of the female translocation carriers

Animal code	Mean litter size[a]	F_1Translocation carriers / male progeny	Chromosomes involved	Presumed break points[b]
First experiment				
AAD-24	4.0	3/6	T(6;11)	6D1; 11A3.2
AAD-34	2.6	2/2	T(1;4)	1B; 4D3
AAD-45	4.0	2/6	T(5;17)	5F; 17B2
AAD-50	4.2	4/8	T(12;17)	12D1; 17D
AAD-52	4.7	3/5	T(11;14)	11B3; 14D3
AAD-58	3.9	1/3	T(4;8)	4E2; 8C1
AAD-101	2.5	3/5	T(1;9)	1F; 9D
Second experiment				
AAD-151	3.0	2/5	T(2;11)	2B; 11D
AAD-161	2.5	3/3	T(4;10;15)	nd
AAD-168	4.0	2/3	T(5;14)	5F; 14D1
AAD-189	4.0	1/3	T(12;18)	12E/F; 18C
AAD-196	4.0	3/4	T(3;5)	3F2; 5E4
AAD-219	3.3	2/6	T(7;17)	7F3; 17B1

[a] From 3-5 litters.
[b] nd = not determined.

+ T[5;10], mean testis weight 20.1 mg) and one carried a complex translocation involving three different chromosomes (AAD-165: T[14;17;19], mean testis weight 27.5 mg) which is shown in Fig. 2B. Two sterile males in the second experiment carried a translocation between chromosome 9 and the Y chromosome whereby the presumed breakpoints on the Y chromosomes were not identical (AAD-181: YD and AAD-227: YC2). Their mean testes weights were 39.2 and 28.7 mg, respectively, and their spermatocytes at diakinesis were characterized by a high rate of XY univalents (81 and 64%, respectively). The remaining four sterile males carried simple reciprocal translocations, their testes weights ranged between 16.5 and 41 mg, and only one showed a long marker chromosome (AAD-127: T[7;10]). The testes weights of semi-sterile males ranged from 70 to 90 mg and that of normal adult males ranged from 90 to 120 mg. One male that carried two independent reciprocal translocations (testes weight 66.6 mg) sired one litter of two female offspring and died before karyotyping could be performed. It is noteworthy that two of the semi-sterile males carried translocations involving chromosomes 4 and 8, however, with different breakpoints on both chromosomes (AAD-198: 4C6 and 8E1; AAD-236: 4B3 and 8D3), which indicates that they were induced independently.

Table 4 shows the characteristics of the identified female translocation carriers. None of them was sterile even though one carried a complex translocation involving three chromosomes (AAD-161: T[4;10;15], Fig. 2A). The break points for this translocation could not be identified in the M-FISH analysis.

The detailed analysis of the translocation break points allows two conclusions. First, none of the translocations observed was identical to another one, i.e. they were not pre-existing in the mouse stock. Second, there were no hotspots for AA-induced chromosome breakage since the break points were randomly scattered among chromosomes (1–8 breaks per chromosome) and along chromosome axes even though location of breaks in light bands prevailed. Most likely, that is due to the process of karyotyping which is oriented at the displacement of dark bands.

The total translocation frequency after dermal exposure was 8.63% and compared to 21.9% after ip exposures to 5 × 50 mg/kg of AA (Adler, 1990) yields a calculated ratio of ip vs. dermal of 0.39 (8.63:21.9). This ratio pertains only to external exposures and observed biological outcomes (reciprocal translocations). Internal exposures, i.e. plasma or testes levels of AA were not measured. The factor 0.39 should be applied to correct the genetic risk calculation based on ip exposure (Adler et al., 1994) in order to derive a more realistic estimate for human dermal exposures. However, there are two caveats. First, the ip treated males were mated 7–11 days after the end of treatment while the males treated dermally were mated 1.5–8.5 days after the end of treatment, i.e. the exposed germ cell stages were overlapping but not identical, and second, the males exposed dermally were housed individually but were not prevented by collars to lick some of the solutions applied to their backs. Therefore, they may have been exposed dermally as well as orally, the latter to an unknown extent. Yet, AA workers in Chinese family-run factories without any hygienic measures are exposed also through the skin and by ingestion. Even though a

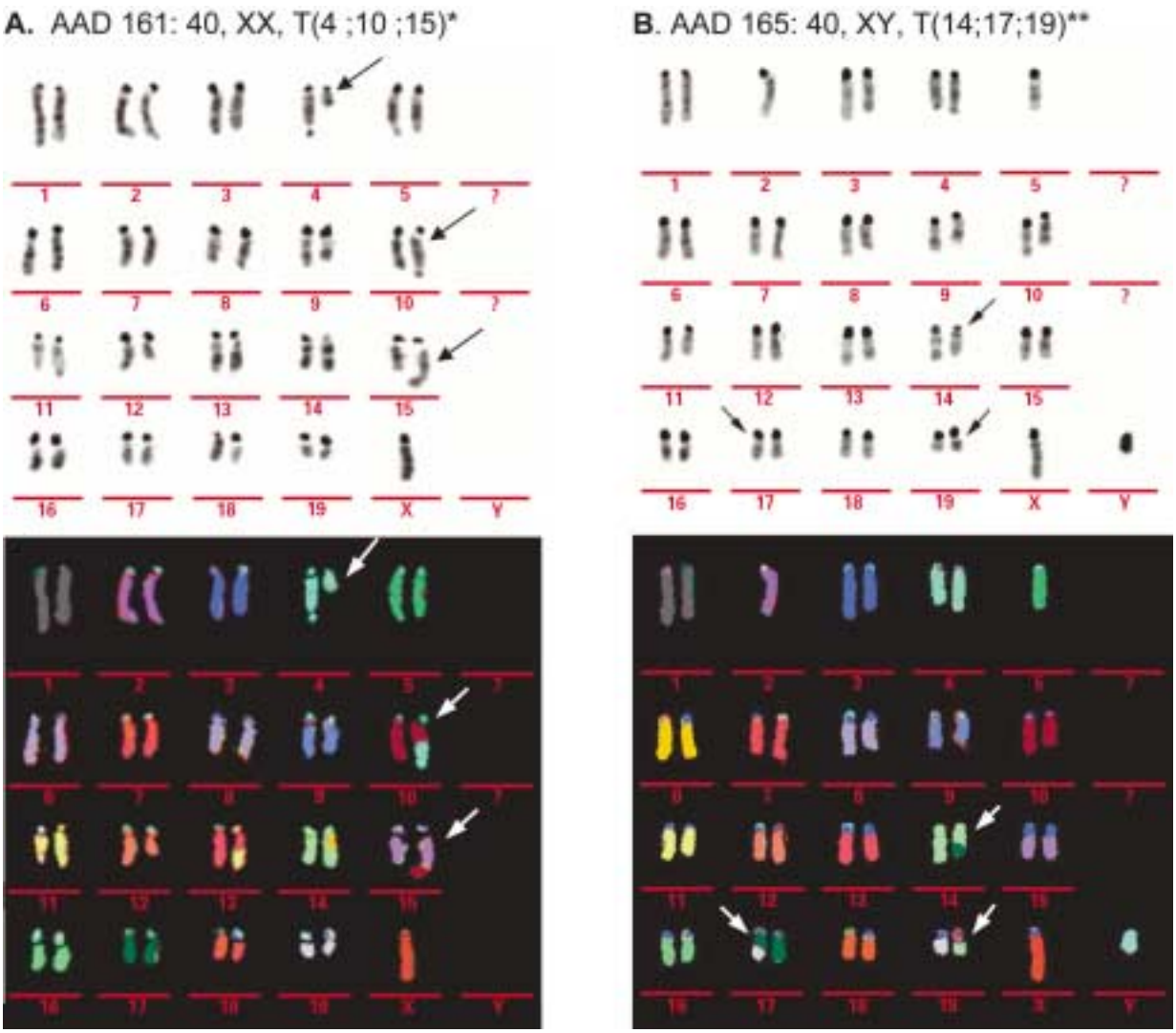

Fig. 2. G-banded and M-FISH karyotypes of mice with complex translocations induced by dermal exposure to acrylamide. The notations of the karyotypes refer to the chromosome constitution of the animals not the cells shown. (**A**) AAD 161: 40, XX, T(4 ;10 ;15); one X chromosome is missing in this cell. (**B**) AAD 165: 40, XY, T(14;17;19); one chromosome 2 and one chromosome 5 are missing in this cell.

linear dose-response could be demonstrated for AA-induced micronuclei in mouse bone marrow cells (Abramsson-Zetterberg, 2003), the extrapolation from linear dose-response at high acute experimental exposure to chronic human exposure with low doses is a matter of debate and ideally should be verified experimentally. Unfortunately, no animal facility is large enough to allow the appropriate heritable translocation experiments. Thus, using the correction factor for ip vs. dermal exposure provides as close a risk estimate as we can presently supply.

By using the linear extrapolation, it was generally assumed that the low-dose effect would be overestimated and an error would be made on the safe side. However, a recent publication alerts to a possible underestimate by this procedure (Witt et al., 2003). For N-hydroxymethylacrylamide (NHMA), these authors demonstrated that high acute ip treatments (MTD 1 × 150 or 5 × 50 mg/kg daily) or subchronic oral treatments (42–168 mg/kg daily for 31 days) did not induce dominant lethal mutations. In contrast, 13 weeks of exposure of male mice to drinking water containing 180–720 ppm NHMA concentration-dependently increased the frequencies of pre- and postimplantation embryonic losses. The concurrent studies of absorption, distribution, metabolism and elimination of ^{14}C-NHMA suggested that bioaccumulation might be responsible for the dominant lethal effects in the long-term drinking water study. The applied bioaccumulation model also indicated that the total dose rather than the duration or route of exposure determined the genetic responses in male germ cells. Although these studies were performed with relatively high doses, the results point to the necessity of animal experiments with chronic exposures for the detection of germ cell mutagens and for the improvement of genetic risk quantification.

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