Mutagenesis vol.7 no.2 pp.145–149, 1992

# Genotoxicity of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and related compounds in Drosophila

**Ulrich Graf, Dieter Wild[1] and Friedrich E.Würgler**

Institute of Toxicology, Swiss Federal Institute of Technology and University of Zurich, PO Box 550, CH-8603 Schwerzenbach, Switzerland and [1]Institute of Pharmacology and Toxicology, University of Würzburg, D-8700 Würzburg, FRG

**The potent food mutagen and carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and the structurally related heterocyclic aromatic amines 2-aminoimidazo[4,5-*f*]quinoline (demethyl-IQ) and 2-amino-1-methylimidazo[4,5-*f*]quinoline (iso-IQ) were assayed for genotoxicity in the wing somatic mutation and recombination test (SMART) as well as in the sex-linked recessive lethal (SLRL) test in *Drosophila melanogaster*. In addition, 3-methyl-2-nitroimidazo[4,5-*f*]-quinoline (nitro-IQ), 2-nitrofluorene and 1,8-dinitropyrene were also assayed in the wing spot test. IQ was clearly mutagenic in the SLRL test with highest activity in spermatids. Iso-IQ was more active than IQ whereas demethyl-IQ was inactive in this test. The same pattern of results was obtained in the wing SMART: iso-IQ produced >2-fold higher frequencies of spots than IQ and demethyl-IQ was clearly negative. In addition, nitro-IQ exhibited an approximately equal genotoxic activity as IQ. 2-Nitrofluorene and 1,8-dinitropyrene were both inactive in the wing spot test. These data provide good evidence for a correlation of genotoxic effects in germinal and somatic cells, and for the practical advantage of the wing spot test in Drosophila. Moreover, the results show structure–activity relationships among the heterocyclic aromatic amines and nitro compounds similar to those found in Salmonella.**

## Introduction

Heterocyclic aromatic amines (amino-imidazoarenes) occurring in fried meat represent a new class of supermutagens in the Salmonella/microsome test. They are also carcinogens in mice and rats and are therefore suspected to contribute to diet-related human tumour risk (Sugimura *et al.*, 1988). Their potential role for carcinogenic as well as genotoxic effects in man and their outstanding mutagenic activity in Salmonella make an assessment of their genotoxicity in higher organisms mandatory. Drosophila offers the advantage to assay genotoxic effects in germ cells and in somatic cells: the test for sex-linked recessive lethal (SLRL) mutations in male germ cells has been widely used (Lee *et al.*, 1983), and more recently the wing or eye spot tests for somatic mutation and mitotic recombination (SMART) have been introduced and validated (Graf *et al.*, 1984, 1989; Würgler and Vogel, 1986; Vogel, 1987).

Heterocyclic as well as 'classical' aromatic amines require oxidative metabolic activation by mammalian microsomal enzymes to express their mutagenic activity in Salmonella (Kato *et al.*, 1983). In Drosophila, numerous procarcinogens and promutagens can be activated and induce SLRL mutations (Vogel *et al.*, 1980). In particular, the heterocyclic aromatic amine 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) has been shown to be positive in this germ cell assay (Wild *et al.*, 1986). However, several carcinogenic and mutagenic aromatic amines have previously failed to induce SLRL mutations in spermatogenic cells of Drosophila or exhibited only very weak genotoxic effects (for details, see Vogel, 1988). In contrast, the aromatic amines 2-acetylaminofluorene (Graf and Würgler, 1988), 2-aminofluorene (Graf *et al.*, 1990), benzidine (Graf *et al.*, 1989), as well as IQ (Yoo *et al.*, 1985), induced somatic mutations and mitotic recombinations in the wing spot test in Drosophila.

Aromatic nitro compounds are related to the aromatic amines in terms of their genotoxic effects because they can form—after reductive metabolic activation—ultimate mutagens identical to those formed from the matching amines (Dirr and Wild, 1988). For these reasons, IQ and the structurally related compounds 2-amino-1-methylimidazo[4,5-*f*]quinoline (iso-IQ) and 2-amino-imidazo[4,5-*f*]quinoline (demethyl-IQ) were assayed for SLRL mutations. These chemicals were also tested for somatic mutations and mitotic recombinations in the wing spot test together with the three nitro aromatics 3-methyl-2-nitroimidazo[4,5-*f*]quinoline (nitro-IQ), 2-nitrofluorene and 1,8-dinitropyrene.

In this context, the present study addresses the following three problems. (i) What is the performance of *Drosophila melanogaster* and, specifically, the wing spot test compared with the SLRL test for the identification of mutagenic/carcinogenic heterocyclic aromatic amines? (ii) Can Drosophila perform a reductive metabolic activation of a nitro-heterocyclic compound related to IQ and of other nitroarenes? (iii) Is there a correlation between the mutagenicity of heterocyclic aromatic amino and nitro compounds in the two organisms Drosophila and Salmonella, and can conclusions be derived concerning the genotoxic mechanism?

## Materials and methods

### *Chemicals*

IQ (CAS 76180-96-6), demethyl-IQ, iso-IQ and nitro-IQ were synthesized and characterized as previously described (Wild *et al.*, 1985; Kaiser *et al.*, 1986; Dirr and Wild, 1988). These compounds were dissolved in phosphate buffer, pH 5. 2-Nitrofluorene (2-NF; CAS 607-57-8) was obtained from Aldrich-Chemie (Steinheim, FRG) and 1,8-dinitropyrene (1,8-DNP; CAS 42397-65-9) from Sigma (St Louis, MO). These two compounds were dissolved in Tween-80 (Serva, Heidelberg, FRG) together with ethanol (Merck, Darmstadt, FRG). The structural formulae of the six test compounds are shown in Figure 1.

### *Larval feeding*

The test solutions and an equal volume of dry Drosophila Instant Medium (Formula 4-24, Carolina Biological Supply Co., Burlington, NC) were used to prepare medium for larval feeding in small vials. For the negative control treatments the respective solvents were used. For the SMART test 3 day old larvae were collected from a cross of optimally fertile flies (see below) and put into the test vials. The larvae were then fed on the medium for the rest of their development (~48 h).

### *SMART test*

For this test the standard cross was used: *mwh* females crossed to *flr*$^{3}$/*TM3, ri p*$^{p}$ *sep bx*$^{34e}$ *e*$^{s}$ *Ser* males. Detailed information on the genetic markers can be

found in Lindsley and Zimm (1985, 1990). Eggs were collected for 8 h in well-yeasted culture bottles. After 3 days the larvae were collected from the bottles and then fed as described above. The hatching flies of the *trans*-heterozygous

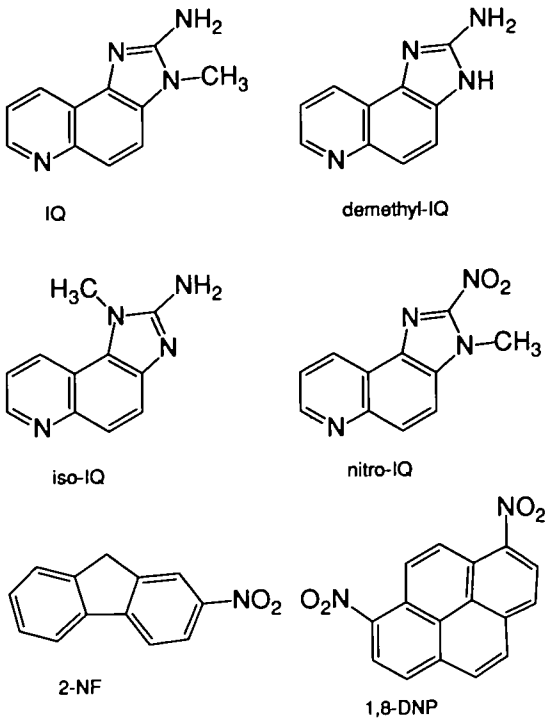


**Fig. 1.** Structural formulae of the test compounds.

(*mwh flr*$^{+}$/*mwh*$^{+}$ *flr*$^{3}$) genotype were collected and stored in 70% ethanol. The subsequent processing and analysis of the wings was carried out as described previously (Graf *et al.*, 1984, 1989; van Schaik and Graf, 1991). The evaluation and statistical analysis of the wing spot data were performed with the computer program SMART (Würgler, unpublished) according to the procedures described elsewhere (Frei and Würgler, 1988; Graf *et al.*, 1989; van Schaik and Graf, 1991; Frei *et al.*, 1992).

*Adult feeding*

For the SLRL assay 1 day old *Berlin K* males were fed for 3 days with 5% (w/v) sucrose solutions in water containing the test chemical. The adult feeding was performed according to the method of Vogel and Lüers using glass filter funnels (Würgler *et al.*, 1984).

*SLRL test*

The treated *Berlin K* wild-type males were crossed individually to three virgin *Basc* females (*Basc* = *In*(1)*sc*$^{S1L}$*sc*$^{8R+S}$, *sc*$^{S1}$ *sc*$^{8}$ *w*$^{a}$ *B*). After 3 days the females were discarded whereas the individual males were again crossed to three new *Basc* females for 3 days. This procedure was repeated once more. The three broods (A, 3 days; B, 3 days; C, 4 days) obtained in this way correspond to cells treated as mature sperm and late spermatids, early spermatids, and late spermatocytes, respectively. The SLRLs were assayed according to standard procedures (Gocke *et al.*, 1981; Würgler *et al.*, 1984). Statistical evaluation was performed according to Frei and Würgler (1988). All the experiments, including the feeding of adult males and larvae, were carried out at 25°C and 60–70% relative humidity.

## Results and discussion

The data collected with the six compounds in the wing SMART test are compiled in Table I. Three concurrent control series were performed with phosphate buffer, 1% Tween-80 plus 5% ethanol and 5% Tween-80 plus 5% ethanol, respectively. The frequencies of spontaneous spots recorded in these series are similar and within the normal control range (Graf *et al.*, 1989; van Schaik and Graf, 1991). Of the four IQ compounds analysed, IQ, iso-IQ

**Table I.** Summary of results obtained in the Drosophila wing SMART test

| Compound and concentration (mM) | No. of wings | Spots per wing (no. of spots)[a] | | | | Spots with *mwh* clone | Mean clone size class | Frequency of clone formation per $10^5$ cells[b] | |
|---|---|---|---|---|---|---|---|---|---|
| | | small single spots (1–2 cells) ($m$ = 2) | large single spots (>2 cells) ($m$ = 5) | twin spots ($m$ = 5) | total spots ($m$ = 2) | | | observed | control corrected |
| Control (phosphate buffer, pH 5) | | | | | | | | | |
| 0 | 200 | 0.26 (52) | 0.03 (5) | 0.04 (7) | 0.32 (64) | 63 | 1.87 | 1.3 | |
| IQ | | | | | | | | | |
| 1.26 | 142 | 0.60 (85)+ | 0.25 (35)+ | 0.15 (21)+ | 0.99 (141)+ | 141 | 2.35 | 4.1 | 2.8 |
| 2.52 | 12 | 0.58 (7)+ | 0.17 (2)i | 0.17 (2)i | 0.92 (11)+ | 11 | 2.09 | 3.8 | 2.5 |
| Iso-IQ | | | | | | | | | |
| 1.26 | 154 | 1.23 (189)+ | 0.64 (98)+ | 0.39 (60)+ | 2.25 (347)+ | 343 | 2.52 | 9.1 | 7.8 |
| 2.52 | 66 | 2.12 (140)+ | 1.02 (67)+ | 0.68 (45)+ | 3.82 (252)+ | 246 | 2.37 | 15.3 | 14.0 |
| Demethyl-IQ | | | | | | | | | |
| 2.73 | 148 | 0.24 (36)– | 0.03 (5)– | 0.02 (3)– | 0.30 (44)– | 44 | 1.77 | 1.2 | –0.1 |
| 5.46 | 62 | 0.26 (16)– | 0.05 (3)i | 0.02 (1)– | 0.32 (20)– | 20 | 1.85 | 1.3 | 0.0 |
| Nitro-IQ | | | | | | | | | |
| 0.51 | 154 | 0.42 (64)+ | 0.12 (19)+ | 0.07 (11)i | 0.61 (94)+ | 91 | 2.16 | 2.4 | 1.1 |
| 1.02 | 150 | 0.62 (93)+ | 0.17 (26)+ | 0.17 (25)+ | 0.96 (144)+ | 144 | 2.16 | 3.9 | 2.6 |
| Control (1% Tween-80 + 3% ethanol) | | | | | | | | | |
| 0 | 320 | 0.28 (91) | 0.05 (16) | 0.01 (3) | 0.34 (110) | 109 | 1.86 | 1.4 | |
| 2-NF | | | | | | | | | |
| 12.5 | 80 | 0.14 (11)– | 0.01 (1)– | 0.03 (2)i | 0.17 (14)– | 14 | 1.79 | 0.7 | –0.7 |
| 25 | 120 | 0.28 (33)– | 0.05 (6)– | 0.04 (5)+ | 0.37 (44)– | 44 | 1.91 | 1.5 | 0.1 |
| Control (5% Tween-80 + 5% ethanol) | | | | | | | | | |
| 0 | 302 | 0.24 (71) | 0.04 (13) | 0.02 (6) | 0.30 (90) | 87 | 2.05 | 1.2 | |
| 1,8-DNP | | | | | | | | | |
| 2 | 139 | 0.22 (30)– | 0.06 (8)– | 0.01 (1)– | 0.28 (39)– | 37 | 2.14 | 1.1 | –0.1 |

[a]Statistical diagnoses according to Frei and Würgler (1988) for comparisons with corresponding controls: + = positive; – = negative; i = inconclusive. $m$ = multiplication factor. Kastenbaum–Bowman tests, one-sided. Probability levels: $\alpha = \beta = 0.05$.
[b]Frequency of clone formation: *mwh* clones/wings/24 400 cells (without size correction).

and nitro-IQ were clearly genotoxic, giving positive results for all three categories of spots. From the positive outcomes obtained for the twin spots it can be concluded that these three compounds have recombinogenic activity. In contrast, demethyl-IQ was non-genotoxic giving negative results for the three categories of spots (see Table I and Figure 5). With respect to the quantitative aspects, it is obvious that iso-IQ is more genotoxic than IQ and nitro-IQ. This is best illustrated by the spot size distributions for single and twin spots recorded after treatment with 1.26 mM IQ and iso-IQ, respectively, and 1.02 mM nitro-IQ (see Figures 2–4). For a quantitative comparison of the four IQ compounds,

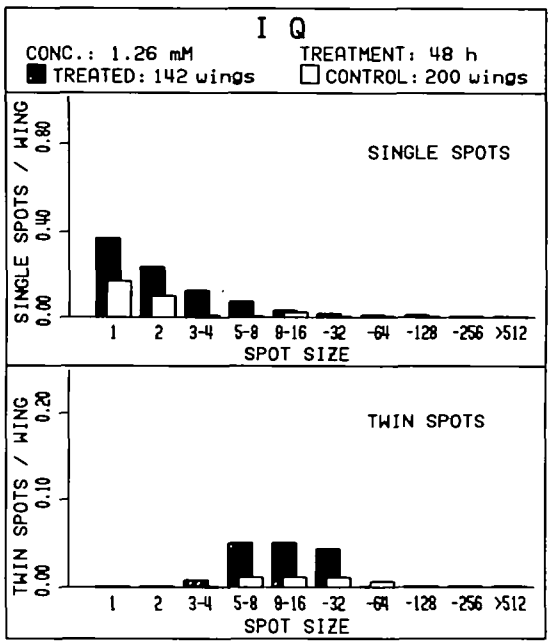


Fig. 2. Spot size distributions for single and twin spots recorded with 1.26 mM IQ.

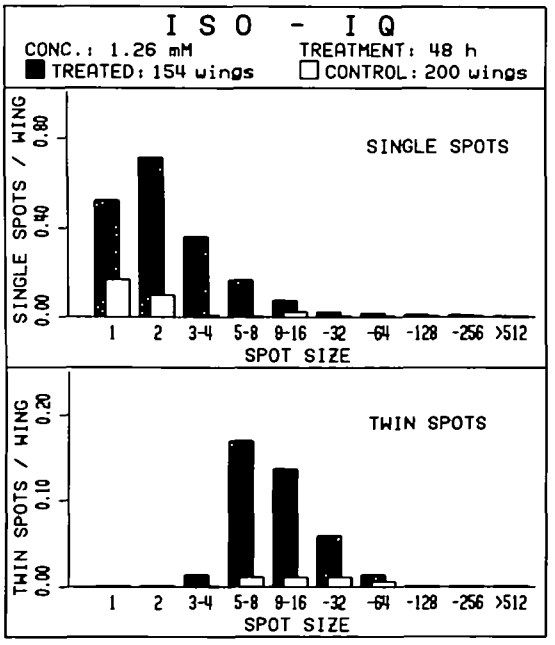


Fig. 3. Spot size distributions for single and twin spots recorded with 1.26 mM iso-IQ.

the frequencies of spots recorded can be converted to frequencies of spots/wing/mM. These values, calculated for single spots and twin spots separately, are plotted in Figure 5. For the three positive compounds IQ, iso-IQ and nitro-IQ, the frequencies of total spots/wing/mM are 0.79, 1.79 and 0.94, respectively. This corresponds to a ratio of 1:2.3:1.2. In other words, iso-IQ is more than twice as genotoxic as IQ and nitro-IQ. A similar conclusion can be drawn when the control-corrected frequencies of clone formation per $10^5$ cells given in Table I are compared: for 1.26 mM IQ and iso-IQ these values are 2.8 and 7.8, respectively, and for 1.02 mM nitro-IQ the value is 2.6. From these values a ratio of 1:2.8:0.9 is obtained which is in good agreement with that determined above.

In contrast to the heterocyclic aromatic nitro compound nitro-IQ, the two nitro compounds 2-NF and 1,8-DNP are non-genotoxic under the same test conditions. The one positive result obtained for the twin spots with 25 mM 2-NF is most probably just due to chance variations. Presumably, these two compounds are not activated into genotoxic metabolites as easily as is nitro-IQ; or alternatively the metabolites are much less

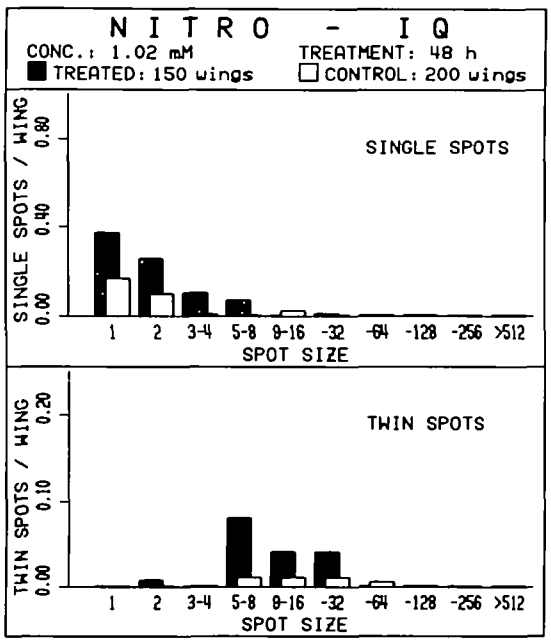


Fig. 4. Spot size distributions for single and twin spots recorded with 1.02 mM nitro-IQ.

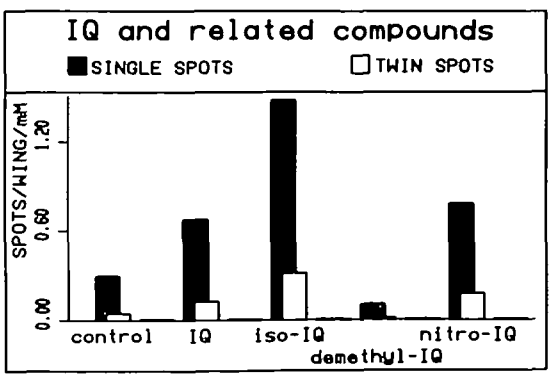


Fig. 5. Frequencies of single spots and twin spots/wing/mM determined for the four IQ compounds.

Table II. Results obtained in the Drosophila SLRL test

| Compound and concentration (mM) | SLRL mutations/chromosomes (%)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | brood A | | brood B | | brood C | | total | |
| IQ[b] | | | | | | | | |
| 1.0 | 18/3485 | (0.52)+ | 23/3250 | (0.71)+ | 15/3480 | (0.43)i | 56/10215 | (0.55)+ |
| 2.5 | 4/1098 | (0.36)i | 9/964 | (0.93)+ | 2/1006 | (0.20)i | 15/3068 | (0.49)+ |
| 4.0 | 6/1097 | (0.55)i | 14/1140 | (1.23)+ | 4/771 | (0.52)i | 24/3008 | (0.80)+ |
| Iso-IQ | | | | | | | | |
| 5.0 | 9/1155 | (0.78)+ | 38/1179 | (3.22)+ | 9/1020 | (0.88)+ | 56/3354 | (1.67)+ |
| Demethyl-IQ | | | | | | | | |
| 5.0 | 11/2442 | (0.45)i | 1/2434 | (0.04)− | 7/2423 | (0.29)i | 19/7299 | (0.26)− |
| Historic control[c] | | | | | | | | |
| 0.0 | 37/15632 | (0.24) | 30/15161 | (0.20) | 39/14880 | (0.26) | 106/45673 | (0.23) |

[a]Statistical diagnoses according to Frei and Würgler (1988): +, positive; −, negative; i, inconclusive. Multiplication factor: $m = 2$. Kastenbaum−Bowman tests, one-sided. Probability levels: $\alpha = \beta = 0.05$.
[b]Data from Wild *et al.* (1985).
[c]Data from Gocke *et al.* (1982).

reactive. The activity of 2-NF was also very weak in Salmonella (strain TA98), whereas nitro-IQ was several orders of magnitude more powerful (Dirr and Wild, 1988). It is very likely that the activation of nitro-IQ and other nitroaromatic compounds is due to a reductive metabolism. It is nevertheless of interest to determine whether the use of the 'improved high bioactivation' cross, which is characterized by a high capacity to activate cytochrome P450-dependent promutagens (Graf and van Schaik, 1992), will help to detect a genotoxic activity of 2-NF and 1,8-DNP in somatic cells of Drosophila.

SLRL tests were carried out with the three chemicals IQ, iso-IQ and demethyl-IQ. The results are shown in Table II. IQ is positive at all three concentrations tested; however, the effect is restricted mainly to brood B which corresponds predominantly to germ cells treated as spermatids. The spermatid stage is thus most sensitive to the genotoxic effects of IQ. In contrast to IQ, iso-IQ gave positive results in all three broods showing again the highest activity in brood B. For brood B the frequencies of SLRL are 1.23 for 4 mM IQ and 3.22 for 5 mM iso-IQ. Assuming parallel dose−response curves for the two compounds, the correction for the different concentrations leads to a ratio of 1:2.1. For the total of all broods this same ratio is 1:1.7. Demethyl-IQ which was tested at 5 mM concentration is clearly negative in the SLRL test. The ratio of the relative mutagenicities found in germ cells corresponds extremely well with the one established with these compounds in the somatic cells of the wing. This is further proof for the equal or even slightly higher sensitivity of the somatic test systems compared to the germ cell assays (Graf and Würgler, 1988; Vogel, 1988; Frei *et al.*, 1992). Given the fact that the somatic tests are less time consuming and therefore much more efficient than the germ cell tests, it is justified to recommend a change in test strategy. In Drosophila, new compounds should first be analysed in the SMART tests. Only if there is a special need should germ cell tests then be carried out (see also Vogel, 1987).

With respect to the structure−activity relationships which can be derived from the results obtained in the Drosophila genotoxicity assays for these compounds, the following conclusions can be drawn. (i) The methyl group present in the imidazole ring of the IQ compounds is required for genotoxic activity. (ii) The methyl group in position 1 (iso-IQ) is >2-fold more efficient than the methyl group in position 3 (IQ). (iii) Substitution of the amino group in the imidazole ring by a nitro group (nitro-IQ) does not alter the genotoxic activity. These relationships are similar to those found in the Salmonella/microsome test using strain TA98 (Nagao *et al.*, 1981; Wild *et al.*, 1985, 1986; Kaiser *et al.*, 1986; Dirr and Wild, 1988). (iv) Although nitro-IQ can be activated very efficiently, 2-NF and 1,8-DNP apparently cannot. These negative results are in contrast to the weak genotoxic activity obtained with the corresponding amino (Graf *et al.*, 1990) or acetylamino (Graf and Würgler, 1988) derivatives of fluorene. This basic difference may be a reflection of the extremely high genotoxic potency of the ultimate mutagens, aryl nitrenium ions, of IQ and related compounds (Wild and Dirr, 1989; Wild, 1990).

In conclusion, we have shown that the wing SMART test in Drosophila is an efficient system for the study of structure−activity relationships and is ideally suited to detect the genotoxic activity of IQ and related compounds.

## Acknowledgements

We thank Eveline Holliger, Ottavina Lutz and Doris Singer for dedicated technical assistance.

## References

Dirr,A. and Wild,D. (1988) Synthesis and mutagenic activity of nitroimidazoarenes. A study on the mechanism of the genotoxicity of heterocyclic arylamines and nitroarenes. *Mutagenesis*, **3**, 147−152.

Frei,H. and Würgler,F.E. (1988) Statistical methods to decide whether mutagenicity test data from Drosophila assays indicate a positive, negative or inconclusive result. *Mutat. Res.*, **203**, 297−308.

Frei,H., Clements,J., Howe,D. and Würgler,F.E. (1992) The genotoxicity of the anti-cancer drug mitoxantrone in somatic and germ cells of *Drosophila melanogaster*. *Mutat. Res.*, in press.

Gocke,E., King,M.-T., Eckhardt,K. and Wild,D. (1981) Mutagenicity of cosmetics ingredients licensed by the European Communities. *Mutat. Res.*, **90**, 91−109.

Gocke,E., Eckhardt,K., King,M.-T. and Wild,D. (1982) Some statistical aspects of spontaneous sex-linked recessive lethal mutations in Drosophila. *Mutat. Res.*, **104**, 239−242.

Graf,U. and Würgler,F.E. (1988) The sex-linked recessive lethal assay and somatic mutation and recombination test in *Drosophila melanogaster*. In Ashby,J., de Serres,F.J., Shelby,M.D. *et al.* (eds), *Evaluation of Short-Term Tests for Carcinogens (International Programme on Chemical Safety's Collaborative Study on in vivo Assays)*. Cambridge University Press/WHO, Cambridge, Vol. 2, pp. 2.301−2.309.

Graf,U. and van Schaik,N. (1992) Improved 'high bioactivation' cross for the wing somatic mutation and recombination test in *Drosophila melanogaster*. *Mutat. Res.*, in press.

Graf,U., Würgler,F.E., Katz,A.J., Frei,H., Juon,H., Hall,C.B. and Kale,P.G.

(1984) Somatic mutation and recombination test in *Drosophila melanogaster*. *Environ. Mutagenesis*, **6**, 153–188.

Graf,U., Frei,H., Kägi,A., Katz,A.J. and Würgler,F.E. (1989) Thirty compounds tested in the Drosophila wing spot test. *Mutat. Res.*, **222**, 359–373.

Graf,U., Hall,C.B. and van Schaik,N. (1990) On the use of excision repair defective cells in the wing somatic mutation and recombination test in *Drosophila melanogaster*. *Environ. Mol. Mutagen.*, **16**, 225–237.

Kaiser,G., Harnasch,D., King,M.-T. and Wild,D. (1986) Chemical structure and mutagenic activity of aminoimidazoquinolines and aminonaphthimidazoles related to IQ. *Chem.-Biol. Interact.*, **57**, 97–106.

Kato,R., Kamataki,T. and Yamazoe,Y. (1983) *N*-Hydroxylation of carcinogenic and mutagenic aromatic amines. *Environ. Health Perspect.*, **49**, 21–25.

Lee,W.R., Abrahamson,S., Valencia,R., von Halle,E.S., Würgler,F.E. and Zimmering,S. (1983) The sex-linked recessive lethal test for mutagenesis in *Drosophila melanogaster*: a report of the US Environmental Protection Agency Gene-Tox Program. *Mutat. Res.*, **123**, 183–297.

Lindsley,D.L. and Zimm,G. (1985) The genome of *Drosophila melanogaster*, Part 1: genes A–K. *Drosophila Inf. Serv.*, **63**, 227 pp.

Lindsley,D.L. and Zimm,G. (1990) The genome of *Drosophila melanogaster*, Part 4: genes L–Z, balancers, transposable elements. *Drosophila Inf. Serv.*, **68**, 382 pp.

Nagao,M., Wakabayashi,K., Kasai,H., Nishimura,S. and Sugimura,T. (1981) Effect of methyl substitution on mutagenicity of 2-amino-3-methylimidazo-[4,5-*f*]quinoline, isolated from broiled sardine. *Carcinogenesis*, **2**, 1147–1149.

Sugimura,T., Sato,S. and Wakabayashi,K. (1988) Mutagens/carcinogens in pyrolysates of amino acids and proteins and in cooked foods: heterocyclic aromatic amines. In Woo,Y.T., Lai,D.Y., Arcos,J.C. and Argus,M.F. (eds), *Chemical Induction of Cancer*. Academic Press, San Diego. Vol. IIIC, pp. 681–710.

van Schaik,N. and Graf,U. (1991) Genotoxicity evaluation of five tricyclic antidepressants in the wing somatic mutation and recombination test in *Drosophila melanogaster*. *Mutat. Res.*, **260**, 99–104.

Vogel,E.W. (1987) Evaluation of potential mammalian genotoxins using Drosophila: the need for a change in test strategy. *Mutagenesis*, **2**, 161–171.

Vogel,E.W. (1988) Summary report on the performance of the Drosophila assays. In Ashby,J., de Serres,F.J., Shelby,M.D. *et al.* (eds), *Evaluation of Short-Term Tests for Carcinogens (International Programme on Chemical Safety's Collaborative Study on in vivo Assays)*. Cambridge University Press/WHO, Cambridge, Vol. 2, pp. 2.277–2.285.

Vogel,E.W., Blijleven,W.G.H., Klapwijk,P.M. and Zijlstra,J.A. (1980) Some current perspectives of the application of Drosophila in the evaluation of carcinogens. In Williams,G.M., Kroes,R., Waaijers,H.W. and van de Poll,K.W. (eds), *The Predictive Value of Short-Term Screening Tests in Carcinogenicity Evaluation* . Elsevier, Amsterdam, pp. 125–147.

Wild,D. (1990) A novel pathway to the ultimate mutagens of aromatic amino and nitro compounds. *Environ. Health Perspect.*, **88**, 27–31.

Wild,D. and Dirr,A. (1989) Mutagenic nitrenes/nitrenium ions from azido-imidazoarenes and their structure–activity relationships. *Mutagenesis*, **4**, 446–452.

Wild,D., Gocke,E., Harnasch,D., Kaiser,G. and King,M.-T. (1985) Differential mutagenic activity of IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline) in *Salmonella typhimurium* strains *in vitro* and *in vivo*, in Drosophila, and in mice. *Mutat. Res.*, **156**, 93–102.

Wild,D., Kaiser,G., King,M.-T. and Harnasch,D. (1986) Genotoxic activity of IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline) and structural analogs. In Knudsen,I. (ed.), *Genetic Toxicology of the Diet*. Alan R.Liss, New York, pp. 145–154.

Würgler,F.E. and Vogel,E.W. (1986) *In vivo* mutagenicity testing using somatic cells of *Drosophila melanogaster*. In de Serres,F.J. (ed.), *Chemical Mutagens, Principles and Methods for their Detection*. Plenum Press, New York. Vol. 10, pp. 1–72.

Würgler,F.E., Sobels,F.H. and Vogel,E. (1984) Drosophila as assay system for detecting genetic changes. In Kilbey,B.J., Legator,M., Nichols,W. and Ramel,C. (eds), *Handbook of Mutagenicity Test Procedures*, 2nd edn. Elsevier, Amsterdam, pp. 555–601.

Yoo,M.A., Ryo,H., Todo,T. and Kondo,S. (1985) Mutagenic potency of amino acid pyrolysates in the Drosophila wing spot test and its correlation to carcinogenic potency. *Gann*, **76**, 468–473.

*Received on October 21, 1991; accepted on November 26, 1991*