

of mutagens with different modes of action are detected by the wing assay. Cross linking agents such as mitomycin C and Trenimon; frameshift mutagens such as acridine orange as well as low molecular aldehydes such as formaldehyde are genotoxic in the assay. Non-disjunction is detected by the wing spot test since vinblastine, a spindle poison interfering with tubulin assembly, induces single spots but not twin spots. Methotrexate, an antimetabolite, is a strong genotoxin in this assay inducing wing spots at high frequencies (Graf *et al.*, 1984).

Strong, direct-acting mutagens such as methylating and ethylating mono-functional alkylating agents are also readily detected in the wing spot test. The following promutagens activated via various metabolic pathways proved to be active in SMART: cyclophosphamide, seneciophylline, procarbazine, DMN, and AFB1. Unstable mutagenic compounds are also easily detected in the wing assay (Graf *et al.*, 1984).

By 1986, some 120 compounds had been tested in the wing spot test. The compounds belong to 27 different chemical classes and include at least fifty compounds that needed metabolic activation. Furthermore, SMART has also been used successfully for the screening of complex mixtures such as herbal teas, coffees and extracts from medicinal plants. For a total of 76 compounds tested in SMART, data were also available for the SLRL assay and, with the majority of compounds, the results were similar in the two test systems. The following five compounds have proved positive in the somatic cell but were negative in the germ cell system: cytosine arabinoside (Katz, 1985),

hydralazine (Juon, unpublished), methotrexate (Würgler *et al.*, 1983), safrole (Würgler *et al.*, 1985) and strychnine (Kägi, unpublished) (in Graf and Würgler, 1986).

SMART tests with *Drosophila* are easy to perform, and what is important for their use in less developed countries is that they do not need expensive laboratory equipment nor sophisticated items. Extensive validation and calibration studies have been performed in three different laboratories during the last ten years (Vogel and Würgler, 1990, unpublished). The Vogel-Würgler (1990, unpublished) table summarizes the test results obtained for 330 chemicals, including some complex mixtures. The file includes all the published and unpublished data from the three laboratories.

10.3.3.2 The Advantages of the SMART assay

Since the essential changes that make cells cancerous are now thought to be primarily somatic mutations (the somatic mutation theory of cancer), the advantages of using this somatic cell assay for screening the environment for potential carcinogens, are manifold. The test detects genotoxins with a broad spectrum of DNA interactions and genetic effects. In addition to individual chemicals, complex mixtures can also be tested for genotoxic activity. This is true for both direct acting agents as well as for compounds requiring metabolic activation. The problem of low detection of polycyclic aromatic hydrocarbons in the SLRL assay in germ cells, has been solved for SMART by the construction of sensitive tester strains with high bioactivation capacity (Frölich and Würgler, 1989).

The somatic assays have been able to detect as mutagens all established *Drosophila* germ line mutagens (mostly tested by the SLRL assay). In addition, classes and individual cases of genotoxins found negative in the germ line assays, such as metabolic inhibitors, were picked up by the SMART assays (Vogel and Würgler, 1990).

By and large, SMART constitutes a broad spectrum assay, both in terms of the different types of genotoxins detected, as well as the different genetic end points scored. The conclusion by Vogel and Würgler (1990), based on analysis of the Gene-Tox report and the outcome of international collaborative studies, was that, when comparing the SLRL assay as a predictor of mammalian genotoxicity with the SMART assay, test performance (including sensitivity and accuracy) of the SMART assays are clearly better than that of the SLRL test.

In 1988, Vogel coordinated a workgroup which considered results from seven laboratories. The ability of four polycyclic hydrocarbons to induce both heritable and somatic genetic alterations in *Drosophila* larvae was assayed. One objective of the experimental procedure was to compile results from germinal versus somatic cells, using the same treatment protocol in larvae. Germ line assays on treated male larvae failed to detect as mutagens any of the four test chemicals (Vogel, 1988). With the four compounds, the testing for somatic mutation and recombination is a more sensitive *in vivo* assay than the SLRL test (Vogel, 1988). The aromatic amines 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* and the improved efficiency of the SMART test compared with the SLRL test to detect the genotoxic activity of the aromatic amine IQ was reported by Graf and colleagues (1992c).

Two collaborative studies on the "Evaluation of Short-Term Tests for Carcinogens" conducted under the auspices of the International programme on Chemical safety with the participation of three laboratories, clearly demonstrated that the SMART assays can successfully be adopted for routine use by other testing laboratories, and that repeated experiments within a laboratory as well as interlaboratory comparisons, give reproducible results. The relative ease with which the assay can be adopted by other *Drosophila* workers and also by genetic toxicologists not familiar with *Drosophila* genetics, has been experienced at three international training courses (Switzerland in 1988, Mexico in 1989 and Brazil in 1990). There are presently 65 laboratories all over the world with the knowledge and means to conduct the SMART assays (Vogel and Würigler, 1990).

Other factors which have contributed to the still increasing acceptance of SMART are the short time needed for test performance, the low costs of the test (10-15% of the SLRL assay) and the flexibility of SMART with respect to variation of exposure conditions and exposure levels. With the SMART test the mutagenic as well as the recombinogenic activity of chemicals can be studied. Experiments have shown that the wing spot test is also suited for the study of dose-response relationships and it lends itself to the analysis of

structure-activity relationships of chemicals (Graf and Würgler, 1986). Such structure-activity relationship studies offer the prospect of identifying the critical parameters in a molecular structure that influence genotoxic and carcinogenic activities. This approach is also of great applied importance because it can facilitate the development of safe chemical products and aid in the search for alternatives to genotoxic compounds whose uses are indispensable.

The SMART test also has some technical advantages. The incredible amount of work involved in preparing thousands of vials and collecting thousands of virgins for the SLRL assay and the heritable translocation test is avoided. The surviving flies can be stored in 70% ethanol until it is convenient to begin mounting, so the slides can be prepared at any time. Furthermore, as the system uses wing tissue, permanent preparations of the wings can be made, making verification and reconsideration possible (Graf *et al.*, 1984) and this allows for re-inspection of critical samples several months later. In my opinion, this fast, *in vivo* eukaryotic short-term test has great potential for use in the genotoxicity screening of chemicals. It is cheap and easy to perform and results can be obtained in under two weeks. The large numbers of experimental animals which can be used, without being considered inhumane by the Animal Ethics Committee, reduces the possibility of missing effects and of other inaccuracies resulting from small sample size, making statistical evaluation of the results possible. One of the main advantages of the SMART test is the fact that large numbers of cells can be scored on the wing blade allowing for the detection of rare events (Graf and Würgler, 1988).

The SMART test detected both the genotoxic and recombinogenic activity of all aflatoxins, sterigmatocystin and zearalenone and the structure-activity series obtained with the aflatoxins was, in decreasing potency, AFB1 > AFG1 > AFB2 > AFG2 which is in agreement with the order previously reported by Wogan and colleagues (1971). Aflatoxin G1 was also tested in the *Salmonella*/mammalian microsome assay, the heritable translocations test and the sex-linked recessive lethals test. In the *Salmonella*/mammalian microsome assay, AFG1 reverted both strains TA98 and TA100 only in the presence of S9 fraction. Aflatoxin G2 and the animal carcinogen zearalenone, however, are negative in the *Salmonella*/mammalian microsome assay (McCann *et al.*, 1975a; Wehner *et al.*, 1978), but positive in SMART, indicating the increased sensitivity of the SMART assay for promutagens. AFG1 was also mutagenic in the SLRL test and induced translocations in the heritable translocations test, but the mutagenic activity of AFG1 was only detected in early spermatids in both tests. These data also provided good evidence for a correlation of genotoxic and chromosome breakage effects of AFG1 in germinal and somatic cells, and for the practical advantage of the wing spot test, but no additional information was gained by performing the other tests together with SMART. Although the powerful promutagen AFG1 exhibited genotoxic activity in all tests, the cost and labour involved in the assays made it clear that SMART is by far the best short-term mutagenicity test available.

When using SMART, it is not necessary to include many other tests in the test battery as not much information can be gained by supplementing this economical and well validated assay with other assays because SMART is

able to detect the full spectrum of genetic effects. A germ cell assay may be included to determine if the test chemical poses a risk to future generations as well as a mammalian short-term test (such as a mammalian tissue culture test) to determine if differences between mammalian and insect metabolism affect the genotoxicity of the compound. Chemicals in the environment should be screened using an *in vivo* short-term test such as SMART and genotoxic chemicals that show consistent mutagenic activity in this short-term test should then be regarded as potential carcinogens due to the mutagenicity/carcinogenicity relationship. Only then should the chemical be tested in a long term rodent carcinogenicity assay in order to label it a mammalian carcinogen and to determine the target organ of such an agent.

10.3.3.3 Improvement of SMART

Most emphasis has been placed on the study of DNA excision repair defective mutants. A problem, however, is that these mutants are often sterile. Furthermore, as shown by Graf and Würgler (1986), and Graf, Hall and van Schaik (1990) the use of strains carrying different DNA repair defects, to improve the detection capacity of the test, is not recommended. Use of phenobarbital to induce the different enzyme activities relevant to xenobiotic metabolism has not been found to increase the sensitivity of the test (Zijlstra *et al.*, 1984; Frölich and Würgler, 1990 a). The best way, so far, to improve the detection capacity of SMART is by using tester strains with high bioactivation capacity. The results of this study showed that the high bioactivation crosses were more efficient than the standard cross in detecting the genotoxic effects of the border line promutagens, AFG2 and AFB2. What

has to be done now is to map some more genes involved in xenobiotic metabolism, to isolate mutants where these genes are amplified or where these enzymes are overproduced and to try to transfer these to the tester strains to make them even more sensitive and to characterize them further metabolically.

Due to the metabolism of xenobiotics in *Drosophila*, results obtained with this insect system are very likely to be relevant for predicting adverse genetic effects in mammals and man. Therefore, positive results in this *Drosophila* test, point to a potential risk for human somatic cells and signal potential genotoxic carcinogens. It is, however, not possible to quantitatively extrapolate screening data from *Drosophila* to man and mammals as possible differences in metabolism must be taken into account.

In 1991, Jowett and colleagues generated transgenic *Drosophila* larvae which express mammalian cytochrome CYP2B1, which is a member of a large gene family encoding several important drug metabolizing enzymes. The gene was fused to a *Drosophila* promoter which confers expression in the larval fat body. The authors used the SMART test to demonstrate that transgenic larvae expressing the mammalian cytochrome P450 are hypersensitive to cyclophosphamide, a procarcinogen activated by the enzyme. Cyclophosphamide caused a highly significant increase in the frequency of total spots in the wings of both the transgenic α CYP2B1 strain and control strains, but the increase was almost two fold higher in the CYP2B1 strain. With the development of this gene-targeted P element-induced mutation, the

authors concluded that it will be possible to generate *Drosophila* strains in which endogenous genes for xenobiotic metabolizing enzymes have been replaced by human genes (Jowett *et al.*, 1991).

The problem with this study is that the authors tested a procarcinogen which could be activated by the endogenous P450 cytochrome enzyme system of *Drosophila*. Instead of cyclophosphamide, the authors should have chosen to test a polycyclic aromatic hydrocarbon that is non-genotoxic in untransformed *Drosophila* strains but positive in transgenic α CYP2B1 strains expressing mammalian cytochrome P450 before they conclude that this does in fact improve the sensitivity and detecting capacity of the test.

CHAPTER 11

11 CONCLUSION

When screening toxic chemicals for genotoxicity in short-term tests it becomes desirable to test them at sub-toxic exposure levels. The problem is that at these non-toxic levels, genotoxic effects will only be detected if the test system is optimally sensitive. The *Drosophila* somatic mutation and recombination wing spot test (SMART) is a convenient and well validated short-term genotoxicity test and in 1989 Frölich and Würigler increased the sensitivity of the test for promutagens by introducing high bioactivation capacity from the *OR-R* DDT resistant strain into the SMART tester strains. The *OR-R* strain carries the *R1* gene at position 2-65,0 and this gene is responsible for the high constitutive expression of cytochrome P450-dependent activities characteristic of this strain. As the activation by cytochrome P450-dependent enzymes is a very relevant pathway for promutagens, the introduction of this high bioactivation capacity into the tester strains would increase the detectability of promutagens. A drawback of the high bioactivation lines is the presence of an irregular whorling of the trichomes on wing blades which interferes with scoring the wing for mutant spots. The gene responsible for this whorling effect, which I have named paisley (*ply*), was shown to be recessive and located around position 90 on chromosome 2 and is thus linked to the *R1* gene responsible for the high bioactivation. In 1992, Graf and van Schaik established and evaluated an improved HB cross producing *ORR* heterozygous individuals. These have a

normal, undisturbed wing hair pattern while exhibiting P450-dependent bioactivation capacity equal to or even slightly higher than that of the original HB cross. Only promutagens that were detected positive even in the standard cross have been tested in the improved HB cross. Although the IHB cross showed an increased sensitivity for these potent promutagens, animal carcinogens, borderline and weak promutagens that are negative in the SMART standard cross but positive in other short-term tests of mutagenicity should be tested in this cross, to show that the cross is in fact an improvement on the standard cross. In this study, the borderline promutagens AFB2 and AFG2 were tested in the improved HB cross, and although the improved HB cross was more sensitive than the standard cross and helped to confirm the genotoxicity and recombinogenic activity of these compounds, AFB2 and AFG2 were also positive in the standard cross at the higher concentrations. The disadvantage of using the hybrid larvae of the IHB cross is that they are heterozygous for the *Rl* and *p/y* genes. Several events can eliminate the wild type paisley gene (*p/y*⁺) resulting in the expression of whorling in certain individuals. Furthermore, tests of DDT resistance (section 3.3) seem to suggest that the *Rl* gene may not be completely dominant and hence *Rl* homozygotes would be more sensitive to the effects of promutagens than *Rl* heterozygotes. Tests of DDT resistance and results of the biochemical assays, as well as the findings of Graf and van Schaik (1992), seem to indicate that the original *ORR;mwh* stock does not carry the *Rl* gene on chromosome two, although it may carry resistance alleles on the first chromosome. Hence, progeny from the original HB cross may also be heterozygous for the *Rl* gene.

It therefore became necessary to construct new HB strains that are homozygous for the *Rl* gene but whorl-free and this was done by separating the *ply* gene from the *Rl* gene. These new SMART HB strains were shown to have higher bioactivation capacity and improved sensitivity for the detection of borderline promutagens than the standard cross and improved HB cross. AFG2 and sterigmatocystin, which were only detected positive at the high concentrations in the other crosses, were positive at sub-toxic exposure levels in the new HB cross. It was interesting to note that the animal carcinogen, zearalenone, was negative at all concentrations in the SMART standard cross but was detected positive at the two higher concentrations by the new HB cross, demonstrating the improved sensitivity of the new HB cross.

Biochemical assays (section 5.3) provided direct evidence that both the newly constructed stocks *ORR;flr³* and *ORR;mwh* overexpressed P450 cytochrome-dependent activities. The pNA demethylase activity in the new HB tester strains and original *ORR;flr³* strain was similar to that in the *OR-R* strain (from which they were constructed) and 8-10 fold higher than in the standard and original *ORR;mwh* tester strains. The pNA demethylase activity in third instar larvae from the new HB cross was also 5 fold higher than in larval microsomes from the standard cross.

The main advantage of the new HB cross is to combine the high bioactivation capacity of *Rl* homozygous individuals, with the ease of scoring the wings using the same criteria as for the standard cross. This cross has, however, been validated with a small number of chemical mutagens and it should

therefore be used in parallel with the standard cross which has been validated with over 330 chemicals so far. Promutagens previously reported negative in *Drosophila* should now be retested using the sensitive new F₂ cross to further characterize the properties of this new SMART cross.

In addition to the characterization and location of the whorling gene, several characteristics and properties of DDT resistance and the *Rl* gene were analysed. The lethal (critical) DDT concentrations of various stocks were determined and it was found that overall males were more DDT resistant than females, larvae were more DDT resistant than adults and that resistance alleles were located on chromosome one, two and three in *QR-R* strains. The maternal effect of the *Rl* gene was investigated and since adult and larval progeny from the cross resistant females to sensitive males were always more resistant than larval and adult progeny from the reciprocal cross, it was concluded that maternal imprinting was probably involved. Cytogenetic analysis of salivary gland chromosomes of *Rl* heterozygotes from various crosses, revealed regions of asynapsis near the right arm of chromosome 2 (2R) and along the X chromosome. The asynaptic region near 2R spanned position 65 where the *Rl* gene is located. This difference in length of *Rl*⁺ and *Rl* genes seemed to suggest that amplification of the *Rl* gene may be involved in insecticide resistance and high bioactivation capacity, but more conclusive molecular evidence is required.

CHAPTER 12

12.1 APPENDIX A

THE *SALMONELLA*/MAMMALIAN MICROSOME ASSAY

Recipes for stock solutions and media

(Adapted from Maron and Ames (1983))

Vogel-Bonner medium E (50X)

Use: Minimal agar

<i>Ingredient</i>	<i>Per litre</i>
Warm distilled H ₂ O (45°C)	670 ml
Magnesium sulphate (MgSO ₄ ·7H ₂ O)	10 g
Citric acid monohydrate	100 g
Potassium phosphate, dibasic (anhydrous) (K ₂ HPO ₄)	500 g
Sodium ammonium phosphate (NaH ₂ NH ₄ (PO ₄ ·4H ₂ O)	175 g

Salts were added in the order indicated to warm water in a 2 litre flask placed on a magnetic stirring hot plate. Each salt was allowed to dissolve completely before adding the next. The volume was adjusted to one litre and then the medium was distributed into two one-litre glass bottles. The medium was autoclaved in the two loosely capped bottles, for 20min at 121°C. When the solutions had cooled, the blue tops were tightened.

0,5 mM histidino/biotin solution

Use: Mutagenicity assay (add 10ml to 100ml of top agar)

<i>Ingredient</i>	<i>Per 250 ml</i>
D-Biotin (F.W.247,3)	30,9 mg
L-Histidine.HCl(F.W.191,7)	24,0 mg
Distilled H ₂ O	250,0 ml

The biotin was dissolved by heating the water to the boiling point. The solution was autoclaved for 20 min at 121°C and the solution was stored in a glass bottle at 4°C.

Top agar

Use: Mutagenicity assay

<i>Ingredient</i>	<i>Per litre</i>
Agar	6 g
sodium chloride (NaCl)	5 g
Distilled H ₂ O	1000 ml

Top agar, containing 0,6% agar and 0,5% NaCl, was autoclaved in 250 ml glass bottles with rubber-lined plastic screw caps (blue tops) in volumes of 100 ml. Before autoclaving, the cap was loosened to prevent pressure from building up, but afterwards the top agar was stored at room temperature with well tightened tops to prevent evaporation. Before use, the cap was loosened

again, the agar was melted in a microwave oven and the bottle was then kept in a water bath.

Ten millilitres of a sterile solution of 0,5 mM L-histidine.HCl/0,5 mM biotin was added to 100 ml of molten agar and mixed thoroughly by swirling. Two millilitre aliquots of top agar were then pipetted aseptically into culture tubes (13 mm x 100 mm) fitted with plastic caps (Belco).

Salt Solution (1,65 M KCl + 0,4M MgCl₂)

Use: S9 mix for mutagenicity assay

<i>Ingredient</i>	<i>Per 500 ml</i>
Potassium chloride (KCl)	61,5g
Magnesium chloride (Mg ₂ Cl ₂ .6H ₂ O)	40,7
Distilled water to final volume of	500ml

The ingredients were dissolved in water and then the solution was autoclaved for 20 min at 121°C. The salt solution was stored in glass bottles in the refrigerator.

0,2 mM sodium phosphate buffer, pH 7,4

Use: S9 mix for mutagenicity assay

Ingredient

Per 500 ml

0,2M sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$)

(13,8g/500ml)

0,2M disodium hydrogen phosphate (Na_2HPO_4)

440ml

(14.2g/500ml)

The pH was tested and if it was lower than 7,4, more 0,2M disodium hydrogen phosphate was added. The buffer was sterilized by autoclaving for 20 min at 121°C.

1M NADP solution (nicotine adenine dinucleotide phosphate)

Use: S9 for mutagenicity assay

Ingredient

Per 5 ml

NADP (F.W. 787,4)

393,7 mg

sterile distilled water

5 ml

The NADP was added to pre-weighed sterile glass tubes with screw caps. The tubes were wrapped with metal foil to protect against light and the tubes were labelled with the correct weight and calculated volume of water to give a 0,1M solution. The tubes of weighed NADP should be placed in a jar with a tight fitting lid. Silica gel dessicant was placed at the bottom of the jar and the jar was stored in a -70°C freezer. When needed for making up the S9 mix, a tube was removed from the jar and the specified amount of water was

added and mixed by vortexing until the NADP had dissolved and then the tube was placed in an ice bath.

1M glucose-6-phosphate

Use: S9 mix for mutagenicity assay

<i>Ingredient</i>	<i>Per 10 ml</i>
Glucose-6-phosphate (G-6-P)	2,82 g
Sterile distilled water	10 ml

Pre-weighed aliquots of glucose-6-phosphate were prepared as described for NADP and stored in dessicated jars in the freezer. Solutions of G-6-P stored in the freezer are stable for 6 months.

S9 mix (rat liver microsomal enzymes and cofactors)

Use: Mutagenicity assay

<i>Ingredient</i>	<i>Per litre</i>	
	<u>Standard S9 mix</u>	<u>High S9 mix</u>
Rat liver S9 (Aroclor-1254-induced)	2,0 ml (4%)	5,0 ml (10%)
MgCl ₂ -KCl salts	1,0 ml	1,0 ml
1 M glucose-6-phosphate	0,25 ml	0,25 ml
0,1 M NADP	2,0 ml	2,0 ml

0,2 M phosphate buffer (pH 7,4)	25,0 ml	25,0 ml
Sterile distilled water	19,75 ml	19,75ml

The ingredients were added in the reverse order indicated above so that the liver was added to a buffered solution. The solution was prepared fresh and kept on ice. All ingredients were chilled before use. Any left over S9 or S9 mix was discarded as S9 should never be refrozen.

Ampicillin solution (8mg/ml)

Use: Tests of ampicillin resistance

Master plates for R-factor strains

<i>Ingredient</i>	<i>Per litre</i>
Ampicillin trihydrate	0,8 g
Sodium hydroxide (0,02N)	100 ml

The solution was stored in a glass bottle at 4°C.

Crystal violet solution (0,1%)

Use: Tests for crystal violet sensitivity (to confirm *rfa* mutation)

<i>Ingredient</i>	<i>Per litre</i>
Crystal violet	0,1 g

Distilled water	100 ml
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This solution was stored at 4°C in a glass bottle with a screw cap. The bottle was wrapped in metal foil to protect against light

Minimal glucose plates

Use: Mutagenicity assay

<i>Ingredient</i>	<i>Per litre</i>
Agar	15 g
Distilled water	930 ml
50X VB salts	20 ml
40% glucose	50 ml

This medium consists of 1,5% agar and 2% glucose in Vogel-Bonner medium E (50X).

The agar (15g) was added to the distilled water in a 2-litre flask. This was autoclaved for 20 min using low exhaust. When the solution had cooled slightly, the 20ml of sterile 50X VB salts and 50ml of sterile 40% glucose were added. A large magnetic stir bar was added to the flask before autoclaving for mixing. After all the ingredients were added and the solution thoroughly stirred, 30ml was poured into each petriplate.

Histidine/biotin plates

Use: Master plates for non R-factor strains

Tests for histidine requirement

<i>Ingredient</i>	<i>Per litre</i>
Agar	15 g
Distilled water	914 ml
50X VB salts	20 ml
40% glucose	50 ml
Sterile histidine.HCl.H ₂ O (2g per 400ml H ₂ O)	10 ml
Sterile 0,5 mM biotin	6 ml

The agar and water were autoclaved and then the sterile 40% glucose, 50X VB salts and histidine were added to the hot agar solution. The solution was allowed to cool slightly and then the biotin was added. After mixing thoroughly, the plates were poured.

Ampicillin plates and ampicillin/tetracycline plates

Use: Master plates for strains carrying the plasmids pKM101 and pKM101 + pAQ1

<i>Ingredients</i>	<i>Per litre</i>	<i>Plate concentration</i>
Agar	15 g	1,5%
Distilled water	910 ml	-
50X VB salts	20 ml	1X
40% glucose	50 ml	2,0%

Sterile histidine.HCl.H ₂ O (2g per 400ml H ₂ O)	10 ml	260 μ M
Sterile 0,5 mM biotin	6 ml	3 μ M
Sterile ampicillin solution (8mg/ml 0,02 N NaOH)	3,15ml	25 μ g/ml
Sterile tetracycline solution (8mg/ml 0,02 N HCl)	0,25ml	2 μ g/ml

The water and agar were autoclaved for 20 min and then the sterile glucose, 50X VB salts and histidine were added to the hot solution. The solution was mixed and allowed to cool to approximately 50°C. The sterile biotin and ampicillin solutions were added aseptically. Tetracycline was added only for use with TA102 which is tetracycline resistant.

The 50X VB salts and 40% glucose solutions were autoclaved separately by autoclaving for 20 min. Histidine and biotin solutions were also autoclaved. Plates to be used for tests of tetracycline and/or ampicillin resistance can be stored for approximately 2 months at 4°C.

Nutrient agar plates

Use: 1. Tests for genotypes

(a) crystal violet sensitivity (*rfa*)

(b) UV sensitivity (*uvrB*)

2. Tests for viability of bacteria

<i>Ingredient</i>	<i>Per litre</i>
Oxoid nutrient broth	25 g
Agar	15 g
Distilled water	1000 ml

The ingredients were added to a 2-litre flask containing a magnetic stir bar and autoclaved for 30 min at low exhaust. The contents were mixed and the plates were poured.

LB agar plates

Use: Growing the strains for lyophilization

<i>Ingredient</i>	<i>Per litre</i>
Bacto tryptone	10 g
Bacto yeast extract	5 g
NaCl	10 g
Agar	15 g
Distilled water	1000 ml

The ingredients were dissolved in water. The pH was adjusted to 7,0 with 1N NaOH (approximately 2ml/l). The ingredients were added to a 2-litre flask containing a magnetic stir bar. The solution was autoclaved for 30 min at 121°C at low exhaust. The solution was mixed and then the plates were poured. The agar is allowed to cool slightly before pouring the plates to avoid condensation.

12.2

APPENDIX B

MAMMALIAN LIVER S9

Prepared by Dr R. Kfir and Miss B. Genthe, Department of Water Technology, C.S.I.R., according to the procedure published by Maron and Ames (1983).

Induction of Rat Liver Enzymes

They used Sprague-Dawley male rats weighing approximately 200g. Aroclor 1254 was diluted in maize oil to a concentration of 200 mg/ml and a single i.p. injection of 500mg/kg was administered to each rat 5 days before they were killed. The rats were given drinking water *ad libitum* and Purina Laboratory Chow until 12 hours before sacrifice when the food (but not the water) was removed. On the fifth day of induction the rats were killed by cervical dislocation.

Removal of liver from rats

The livers were removed aseptically using sterile surgical tools. After the animal was killed, it was placed on its back on an autopsy board and the feet were secured with pins. The fur was swabbed thoroughly with 70% ethanol and the skin was cut using sterile pointed scissors. The skin flaps were folded back and pinned to the autopsy board to avoid getting fur into the abdominal cavity. The muscle layer was swabbed with ethanol and then this layer was cut through and the liver was excised.

Preparation of liver S9 fraction

The preparation was based on the procedure by Garner *et al.* (1972). All steps of the procedure were carried out at 4°C using cold sterile solutions and glassware. The freshly excised livers were placed in pre-washed beakers containing approximately 1ml of chilled 0.1 M KCl per gram of wet liver. After the livers were weighed, they were washed several times in fresh, chilled KCl. Successive washing in KCl are essential to ensure a sterile preparation and to remove haemoglobin which can inhibit the activity of the cytochrome P450 enzymes. The washed livers were transferred to a beaker containing 3 volumes of 0.15 M KCl (3 ml/g wet liver) and were minced with sterile scissors and homogenized (with a Polytron homogenizer). The homogenate was centrifuged for 10 minutes at 9000g and the supernatant (S9 fraction) was decanted and saved.

12.3

APPENDIX C

PROTEIN DETERMINATION

Reagents used in Protein Determination using

Lowry's technique

Reagent A: 10 ml of 2% (w/v) sodium potassium tartar
 (or 0,2 g potassium tartrate + 10 ml water)
 10 ml of 1% (w/v) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
 (or 0,1 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ + 10 ml water)
 1 000 ml of 2% (w/v) Na_2CO_3 on 0,1 M NaOH
 (or 10 g Na_2CO_3 in 500 ml of 0,1 M NaOH)

Reagent B: Folin diluted with 2 volumes of water

Bottles were covered with aluminium foil because both reagents are light sensitive.

12.4

APPENDIX D

FAURE'S SOLUTION

Recipe for Faure's solution

<i>Ingredients</i>	<i>Per 50 ml</i>
gum arabic	30 g
glycerol	20 ml
chloral hydrate	50 g
distilled water	50 ml

The chloral hydrate was dissolved in water and then the glycerol was added and the ingredients mixed. The gum arabic was suspended in a small gauze bag and allowed to dissolve completely. This solution should not be filtered. Faure's solution should be kept in a cool, dark place in the undiluted form. The solution is distributed to small plastic containers and is diluted approximately 1:3 in distilled water and allowed to settle for a few days before use. The solution should be fluid and clear hence the solution is left to stand so that the impurities can settle at the bottom of the container. The container should not be shaken and it is recommended that the tip of the pasteur pipette should not touch the bottom of the container when pipetting Faure's solution onto well slides.

12.5

APPENDIX E

DROSOPHILA MEDIA

Recipe for *Drosophila* culture medium

<i>Ingredients</i>	<i>Per 10 bottles</i>
agar	10 g
water	240 ml
mealie meal	120 ml
water	200 ml
syrup	60 ml
molasses	15 ml
water	400 ml
yeast	5 g
4g Nipazol M (propyl p hydroxybenzoate) in 4ml 70% ethanol (Nipa laboratories Ltd, Great Britain).	

The agar, syrup, molasses and water (240ml + 400ml) were brought to the boil in a large pot. The mealie meal, yeast, and water (200ml) were mixed separately and then added to the boiling mixture. The nipazol was then added to inhibit fungal growth on the medium. About 80 ml medium was then poured into stock culture bottles (volume 400 ml).

For smaller cultures, such as pair matings during the location of the paisley gene crosses and for the heritable translocations and sex-linked recessive

lethals assay, vials (40 ml) were used. These glass tubes are about 7,5 cm high and have a diameter of about 2,5 cm. About 10 ml of medium is poured into each culture vial. The surface of the medium in culture bottles and in vials is allowed to set and dry for 24 hours under a gauze cover to prevent stray flies from laying eggs in the medium. The following day, the surface of the medium is covered with a layer of live yeast. The cultures were stoppered with plastic foam plugs. The yeast is allowed to dry for another 24 hours and the bottles are then ready for use. Vials and stoppers are carefully washed prior to use. Culture bottles are autoclaved before and after use.

New egg-laying medium

This medium is used for the collection of larvae according to the method suggested by Magnusson and Ramel (1990). A solid agar base was made of 5% agar-agar in tap water. The sugar is omitted from the agar base to prevent the larvae from burrowing into the medium. Nipasol is added to the agar and then the agar and water is autoclaved in a large flask for 20 min at 121°C. The flask should be placed in another large container and the flask should be stoppered with cotton wool to prevent the agar from boiling over in the autoclave. The agar base was allowed to dry for twenty four hours and was then completely covered with a thick layer (approximately 5 mm) of a paste of fresh baker's yeast with a little sucrose added. This is allowed to dry under a gauze cover for 24 hours before the introduction of the parents for the egg laying. The agar base bottles without yeast keep well for up to 3 weeks covered with laboratory parafilm (American can company, Greenwich) and kept in the refrigerator at 4°C.

12.6

APPENDIX F

THE SCANNING ELECTRON MICROSCOPE (SEM)

The flies were first killed in a vial by placing cottonwool soaked in acetone over the opening for 5 minutes. Specimens were dried in acetone vapour for two hours by placing cottonwool dabbed with acetone under a circle of filter-paper (Whatman number 1) in a petri dish. The flies were placed on the filter-paper and then the petri dish cover was replaced. After two hours, the wings were removed using fine watchmaker forceps and mounted on SEM aluminium stubs with MIGB conducting glue (a silver paste) (10 wings) and graphite dissolved in ethanol (2 wings). Graphite was very difficult to use because it dries very quickly and there is not enough time to mount the wings. The white glue was spread thinly on the stub, small sections at a time, and then the wings were stuck with the aid of a dissecting microscope.

The specimens were then coated with gold-palladium alloy and after 2 days they were loaded into the Jeol JSM-840 scanning microscope. The acceleration voltage was adjusted to 30 KV and the working distance to 15 mm. The probe current was set at $10 \text{ amp } 10^{-6}$. Brightness and contrast were adjusted and the wings were scanned for mutant spots at a low magnification (550x). When a mutant spot was located, the magnification was increased (up to 10 000x) and the fine focus was adjusted. The stigmators were also used to give the best resolution. Before a photograph was taken, brightness and contrast were again adjusted.

12.7

APPENDIX G

MAINTENANCE OF OR-R STOCKS ON DDT

As directed by Dr Phillip Barnes, Connecticut College, U.S.A.; according to the procedure of Dr Merrell (in Dapkus and Merrell, 1977).

The OR-R stocks were exposed to DDT almost every generation. A stock solution of DDT in acetone (150 mg/ml) was prepared following the same guidelines as for the laboratory use of mycotoxins (Appendix A). A mechanical pipetting aid was used to soak the 25 x 75 mm filter-paper strips (Whatman number 1) with the solution. First 0,5 ml were applied per strip and allowed to dry, and then another 0,5 ml were applied so that a total of 1 ml of DDT solution was applied per strip. If the entire 1 ml is applied at once, then some of it will drip off the strip. After ensuring that the strips were dry, they were added to the culture bottles by placing them at an angle, from one edge of the food bottle interface to the side of the bottle, with the DDT-coated side up. The flies were then transferred to the bottles. The adult flies walk over the strips and eventually settle down on the food surface. The adults lay eggs and then the larvae are exposed to the DDT.

12.8

APPENDIX H

PREPARATION OF SOLUTIONS USED IN CYTOGENETIC OBSERVATION OF
SALIVARY GLAND CHROMOSOMES

Insect Ringer's Solution

<i>Ingredient</i>	<i>Per litre</i>
NaCl	6,5 g
KCl	0,25 g
CaCl ₂	0,3 g
sodium bicarbonate	0,2 g
(approximate to give a pH of 7,4)	
distilled water	1000 ml

Acid-washed coverslips

The coverslips were placed in 1N HCl for twenty minutes and then rinsed in distilled water. They were then soaked in distilled water for ten minutes and then placed and stored in 70% ethanol.

Propio Carmine

<i>Ingredient</i>	<i>Per 100 ml</i>
glacial propionic acid	45 ml
distilled water	55 ml
carmine	0,5 g

This was boiled gently for several hours in a reflux condenser. It was then shaken well and filtered when cool.

12.9

APPENDIX I

ABBREVIATIONS

2-acetylaminofluorene	2-AAF
2-aminofluorene	2-AF
aflatoxin B1	AFB1
aflatoxin B2	AFB2
aflatoxin G1	AFG1
aflatoxin G2	AFG2
aflatoxin M1	AFM1
2-amino-3-methylimidazo [4,5-f]quinoline	IQ
benzo [a] pyrene	BP
carbon dioxide gas	CO ₂
cytochalasin B	CYTB
cytochalasin F	CYTF
cytochalasin J	CYTJ
1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane	DDT
deoxynivalenol	DEOX
diacetoxyscirpenol	DIAC
diethylnitrosamine	DEN
dimethylnitrosamine	DMN
dimethyl sulphoxide	DMSO
deoxyribonucleic acid	DNA
ethylnitrosourea	ENU

fumonisin B1	FB1
fumonisin B2	FB2
Johannesburg	JHB
molecular weight	MW
nicotinamide adenine dinucleotide phosphate	NADP
(reduced form)	NADPH
phenobarbitone/phenobarbital (salt)	PB
p-nitroanisole	pNA
polychlorinated biphenyls	PCBs
ribonucleic acid	RNA
roridin A	RORA
South Africa	SA
South African Institute for Medical Research	SAIMR
sterigmatocystin	STM
ultraviolet	UV
United States of America	USA
verrucarin A	VRCA
vinyl chloride	VC
University of the Witwatersrand	WITS
zearalerone	ZEA

12.10

APPENDIX J

TABLE 12.10.1 SOURCES FROM WHICH TESTER STRAINS AND STOCK CULTURES WERE OBTAINED

TESTER STRAIN	SOURCE
TA97 TA98 TA100 TA102	Miss Heidi Grimmer, Department of Biochemistry, University of the Witwatersrand, Johannesburg.
STOCK CULTURE	SOURCE
mwh flr ³ /TM3Ser Basc;Cy/Pm Lego wild type bw st p ^p	Institute of Toxicology, Swiss Federal Institute of Technology and University of Zürich, Schwerzenbach, Switzerland.
Cy/Pm Cy/Pm; Sb/Ser Basc Basc; Cy/Pm pr vg pi mwh Oregon R wild type	Department of Genetics, University of the Witwatersrand.
OR-R	Dr P. Barnes, Connecticut College, U.S.A.
ORR;mwh ORR;flr ³	Dr A. Mälich, Institute of Toxicology, Swiss Federal Institute of Technology and University of Zürich, Schwerzenbach, Switzerland.

BALANCER CHROMOSOMES

First Chromosome Balancers

The *Basc* chromosome carries the easily recognizable genetic markers *Bar* eyes and white apricot. *Bar* produces a narrow eye shape in the homozygous or hemizygous condition and a kidney shaped eye when heterozygous in females. White-apricot flies differ from wild type flies in that the eye pigment is changed to light orange. The *Basc* flies homozygous for *Bar* and white apricot

are thus characterized by narrow-shaped, light orange eyes. In addition to these visible markers, the *Basc* chromosome contains a combination of two scute inversions: the left hand part of sc^{s1L} and the right hand part of sc , covering the entire X chromosome, and a smaller inner inversion *In-S* (Spencer and Stern, 1948) [*Basc*: *In* (1) $sc^{s1L} sc^{8R}$ + *In* (1) $s, sc^{s1} sc^8 w^a B$ (please refer to Lindsley and Grell for a description of the genetic markers)]. All *In* (1) sc^{s1L} and sc^{8R} derivatives are duplicated for sc . All are viable and fertile in hemizygous males and homozygous females.

Second chromosome balancers

Cy (Curly wings) and *Pm* (Plum eye colour) are dominant markers located on the second chromosome. Both are homozygous lethals and in this line, the chromosome carries large complex inversions which act as crossover suppressors.

The majority of second chromosome balancers are based on *In*(2L)*Cy* (= *In*(2L)22D1.2; 33F5-24A1) plus *In*(2R)*Cy* (= *In* (2R) 42 A2,3; 58 A4-B1). Both of these inversions are spontaneous in origin and the dominant Curly mutation is associated with the 22D1.2 breakpoint of *In*(2L)*Cy*. The simplest *Cy* balancer is *In* (2L)*Cy* + *In* (2R)*Cy*.

Other chromosomes useful for balancing chromosome two belong to the Plum family. These inversions act as dominant variegators of brown (2-104,5). Of these, *In* (2LR)*bw*^{IV} = *In* (2LR)*Pm* is perhaps the most useful. It is *In* (2LR) 21 C8-D1-60D1.2 + *In* (2LR) 40F; 59D4 - E1 and normally carries *bw*^{IV}.

Third chromosome balancers

The third multiple (TM) series

The third multiple series of balancers were designed to provide convenient and effective balancing for chromosome three. All except TM1 are marked with alleles of the bithorax complex.

TM3 is one of the most widely used balancers for chromosome three, although it is far from ideal. It is derived from $T(1;3)sc^{280-20}$, a translocation of the tip of the X chromosome (carrying y^+) to the tip of 3L. This translocation was, itself, induced on $In(3LR) sep + In(3R) C; rip sep bx^{34a} e^5$ [$In(3LR) sep = In(3LR) 65E; 85E$]. The product was irradiated to induce three other inversions $In(3LR) 71C; 94 D-F$, $In(3LR) 76C; 93A$, and $In(3LR) 79E; 100C$. TM3 usually carries both Stubble (*Sb*) and Serrate (*Ser*) as dominant markers, although chromosomes with only one of these are available. In the heterozygous form *Sb* leads to bristles on the thorax that are shorter and thicker than normal bristles, while *Ser* leads to wings missing a section of the wing.

TABLE 12.10.2 SOURCES OF CHEMICALS USED

CHEMICAL	SOURCE
GENERAL Acetone Ethanol Glycerol Propionic acid	Central Stores, University of the Witwatersrand, Johannesburg, SA.
S/M M ASSAY* Sodium azide 2-AF Agar Ampicillin trihydrate D-biotin BSA DMSO D-glucose-6-phosphate L-histidine.HCl NADP Nutrient broth S9 Fraction Tetracycline	Miss Heidi Grimmer, Department of Biochemistry, University of the Witwatersrand, Johannesburg. Oxoid Sigma BDH Chemicals, England. Boehringer Mannheim, Germany. Saarchem, SA. Sigma, USA. Sigma, USA. Boehringer Mannheim, Germany. Oxoid Dr Kfir, Department of Water Technology, CSIR, SA. Sigma, USA.
SMART TEST Biofilm CO ₂ gas Chloral hydrate Instant medium Faure's solution Filter paper Gum Arabic Mycotoxins Nipasol Sucrose Tween 80 Yeast	American Can Company, USA. Afrox Saarchem, SA. Carolina Biological Supply Company, USA. Made up according to the recipe in APPENDIX D by the Institute of Toxicology (Switzerland) and R. Hillier, V. Phillips and R. Pacella (WITS, Johannesburg, SA). Whatman Number 1 Saarchem, SA. Drs Vleggaar and Horak, Department of Food Technology CSIR, SA Nipa Laboratories, South Wales, Great Britain. Saarchem, SA. Saarchem, SA. Anchor, Epping, SA.
CYTOGENETICS Carmine Euparal Film	BDH Chemicals, England. BDH Chemicals, England. Fujicolor Super FR100, Japan. Kodak technical pan film 2415, ESTAR-AH base, TP 135-36.
DDT TESTS DDT	Sigma, USA.
BIOCHEMISTRY NADPH pNA Phenobarbital Sodium dithionite	Boehringer Mannheim, Germany. Aldrich Be-Tabs Pharmaceuticals, SA. Saarchem

*The *Salmonella*/mammalian microsome assay

TABLE 12.10.3 SOURCES FROM WHICH EQUIPMENT WERE OBTAINED

EQUIPMENT	SOURCE
Centrifuge, Beckman, model J2-21, rotor type JA20	Beckman
Centrifuge, Beckman, model L5-50, rotor type 50Ti	Beckman
Compound microscope	Olympus, Japan.
Homogenizer	Virtis, Gardiner, N.Y.
Spectrophotometer	Cary model 210, Varian.
Stereo microscope	Olympus, Japan.

CHAPTER 13

13 REFERENCES

- Abelson, P. (1987).
Cancer Phobia. *Science* 237: 473.
- Abrahamson, S.; Würgler, F.E.; de Jongh, C. and Unger Meyer, H. (1980).
How many loci on the X chromosome of *Drosophila melanogaster* can mutate to recessive lethals? *Environmental Mutagenesis* 2: 247-453.
- Adamson, R.H.; Correa, P. and Dalgard, D.W. (1973).
Occurrence of a primary liver carcinoma in a rhesus monkey fed aflatoxin B1. *J. Nat. Cancer Inst.* 50: 549-553.
- Adler, P.N. (1992)
The genetic control of tissue polarity in *Drosophila*. *BioEssays* 14: 735-741.
- Alonso-Moraga, A. and Graf, U. (1989).
Genotoxicity testing of nitroaromatic nitrofurans in the *Drosophila* somatic mutation and recombination test. *Mutagenesis* 4: 105-110.
- Albrecht, C. (1991).
Don't choose to give cancer a chance: up to 80 percent of cancers linked to lifestyle and environment, *Understanding Oncology*, vol. 2, number 2:29-30
- Alpert, M.E.; Hutt, M.S.R. and Davidson, C.S. (1968).
Hepatoma in Uganda. *Lancet* : 1265-1267.
- Alpert, M.E.; Hutt, M.S.R.; Wogan, G.N. and Davidson, C.S. (1971).
Association between aflatoxin content of food and hepatoma frequency in Uganda. *Cancer* 28: 253-260.
- Ames, B.N. (1971).
The detection of chemical mutagens with enteric bacteria. In: *Chemical Mutagens: Principles and Methods for their Detection*, volume 1, chapter 9. Edited by Hollaender, A.. Plenum Press, New York.
- Ames, B.N. (1979).
Identifying environmental chemicals causing mutations and cancer. *Science* 204: 587-593.

- Ames, B.N.; Durston, W.E.; Yamagaki, E. and Lee, F.D. (1973).
Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. *Proc. Natl. Acad. Sci. (U.S.A.)* 70: 2281-2285.
- Ames, B.N. and Gold, L.S. (1990).
Chemical carcinogenesis: too many rodent carcinogens. *Proc. Natl. Acad. Sci. (U.S.A.)* 87: 7772-7776.
- Ames, B.N.; Profet, M. and Gold, L.S. (1990).
Dietary Pesticides 99,99% all natural. *Proc. Natl. Acad. Sci. (U.S.A.)* 87: 7777-7781.
- Ashburner, M. (1989).
Drosophila, a Laboratory Handbook. Cold Spring Harbor Laboratory Press, New York, 1331 pp.
- Ashby, J. and Tennant, R.W. (1983).
Chemical structure, *Salmonella* mutagenicity and extent of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTP. *Mutation Research* 204: 17-115.
- Atherton, D. and Gall, J. (1972).
Salivary gland squashes for *in situ* nucleic acid hybridization studies. *Drosophila Information Service* 49:131-133.
- Auerbach, C. (1962).
Mutation, Part I: Methods, Oliver and Boyd, Edinburgh, pp 20-62.
- Auerbach, C. and Robson, J.M. (1947).
The production of mutations by chemical substances. *Proc. R. Soc. Edinburgh B* 62: 271-283.
- Baars, A.J. (1980).
Biotransformation of xenobiotics in *Drosophila* and its relevance for mutagenicity testing. *Drug Metab. Rev.*, 11: 191-221.
- Bayo, S.; Parkin, D.M.; Koumaré, A.K.; Diallo, A.N.; Ba, T.; Soumaré, S. and Sangaré, S. (1990).
Cancer in Mali 1987-1988. *Int. J. Cancer* 45: 679-684.
- Becker, H.J. (1976).
Mitotic recombination. In: *The Genetics and Biology of Drosophila*. Ashburner, M. and Novitski, E. (eds), vol. 1c, Academic Press, London, pp 1019-1087.
- Bendele, A.M.; Carlton, W.W.; Krogh, P. and Lillehoj, E.B. (1985).
Ochratoxin A carcinogenesis in the (C57BL/6JXC3H)F1 mouse. *J. Natl. Cancer Inst.* 75: 733-739.

- Berry, C. (1988).
The pathology of mycotoxins. *J. Pathol.* 154: 301-311.
- Boorman, G. (1988).
N.T.P. Technical report on the toxicology and carcinogenesis studies of ochratoxin A (Cas N° 303-47-9) F344 in rats (gavage studies), NIH Publication N° 88-2813. National Toxicology Program, Public Health Service, National Institute of Health, United States Department of Health and Human Services.
- Brattsten, L.B.; Holyoke, C.W.; Leeper, J.R. and Raffa, K.F. (1986).
Insecticide resistance: challenge to pest management and basic research. *Science* 231: 1255.
- Brown, C.A. (1982).
Aflatoxin M1 in milk. *Food Technology in Australia* 34: 228-231.
- Bullerman, L.B. (1979).
Significance of mycotoxins to food safety and human health. *J. Fd. Prot.* 42: 65-86.
- Bullerman, L.B. (1981).
Public health significance of molds and mycotoxins in fermented dairy products. *J. Dairy Sci.* 64: 2438-2452.
- Bullerman, L.B. (1986).
Mycotoxins and food safety. A scientific status summary by the Institute of Food Technologists' expert panel on food safety and nutrition. Institute of Food Technologists, Chicago.
- Busby, W.F. and Wogan, G.M. (1984).
Aflatoxins. In: *Chemical Carcinogens*, Searle, C.D. (ed.), ACS monograph 182, Washington D.C. pp 945-1136.
- Butler, W.H. and Barnes, J.M. (1968).
Carcinogenic action of groundnut meal containing aflatoxin in rats. *Food Cosmet. Toxicol.* 6: 135-141.
- Campbell, T.C. and Salamut, L. (1970).
Aflatoxin ingestion and excretion by humans, pp 271-280. In: *Mycotoxins in Human Health*, Purchase, I.F.H. (ed.), MacMillan Ltd., London.
- Cannon, M.; Jimenez, A. and Vazquez, D. (1976).
Competition between trichodermin and several other sesquiterpene antibiotics for binding to their receptor site(s) on eukaryotic ribosomes. *Biochem. J.* 160: 137-145.

- Carlborg, F.W. (1979).
Cancer, mathematical models and aflatoxin. *Food Cosmet. Toxicol.* 17: 159-166.
- Carnaghan, R.B.A. (1965).
Hepatic tumours in ducks fed a low level of toxic groundnut meal. *Nature (London)* 208: 308.
- Carter, S.B. (1967).
Effect of cytochalasins on mammalian cells. *Nature* 213: 261-264.
- Casida, J.E. (1969).
Insect microsomes and insecticide chemical oxidations. In: *Microsomes and Drug Oxidations*, Gillette, J.R.; Canney, A.H.; Cosmides, G.J.; Estabrook, R.W.; Fauts, J.R. and Mannering, G.J. (eds), Academic Press, New York, pp 517-531.
- Chu, K.C.; Patel, K.M.; Lin, A.H.; Tarone, R.E.; Linhart, M.S. and Dunkel, V.C. (1981).
Evaluating statistical analyses and reproducibility of microbial mutagenicity assays. *Mutation Research* 85: 119-132.
- Fauts, J.D.; Howe, A.; Phillips, M. and Todd, N.K. (1988).
The *Drosophila* wing spot test: a comparison of the sensitivity of the different strains. *Mutation Research* 203: 117-123.
- Cohen, S.M. and Ellwein, L.B. (1990).
Cell proliferation in carcinogenesis. *Science* 249: 1007-1011.
- Conger, A.D. and Fairchild, L.M. (1953).
A quickfreeze method for making smear slides permanent. *Stain Technology* 28: 281-283.
- Cook-Mozaffari, P. and Burkitt, D. (1990).
Cancer in Africa, University of Oxford Monograph.
- Croy, R.G.; Essigman, J.M.; Reinhold, J.M.; Wogan, G.N. (1978).
Identification of the principal Aflatoxin B1-DNA adduct formed *in vivo* in rat liver. *Proc. Natl. Acad. Sci. (USA)* 75: 1745-1749.
- Dapkus, J. and Merrell, D.J. (1977).
Chromosomal analysis of DDT resistance in a long-term selected population of *Drosophila melanogaster*. *Genetics* 87: 685-697.
- Darlington, C.D. and La cour, L.F. (1969).
The handling of chromosomes, fifth edition. George Allen and Unwin Ltd., London, 271 pp.

- Demerec, M. (1948).
Mutations induced by carcinogens. Br. J. Cancer 2: 114-117.
- Dichter, C.R. (1984).
Risk estimates of liver cancer due to aflatoxin exposure from peanuts and peanut products. Fd. Chem. Toxic. 22: 431-437.
- Doll, R (1977).
Strategy for detection of cancer hazards to man. Nature (London) 265: 589-596.
- Doll, R.; Muir, C. and Waterhouse, J. (1970).
Cancer incidence in Five Continents, volume II, International Union Against Cancer, Springer-Verlag, Berlin.
- Donahue, E.V.; McCann, J. and Ames, B.N. (1978).
Detection of mutagenic impurities in carcinogens and non-carcinogens by high pressure liquid chromatography and the *Salmonella*/microsome test. Cancer Research 38: 431.
- Ehling, J. (1980).
Induction of gene mutations in germ cells of the mouse. Archives of Toxicology 46: 123-138.
- Epstein, S.S. (1974).
Environmental determinants of human cancer. Cancer Research 34: 2425-2435.
- Essigman, J.M.; Croy, R.G.; Nadzan, A.M.; Busby, W.F. Jr; Reinhold, V.N.; Buchi, G. and Wogan, G.N. (1977).
Structural identification of the major DNA adducts formed by aflatoxin B1 *in vitro*. Proc. Natl. Acad. Sci. (USA) 74: 1870-1874.
- Essigman, J.M.; Barker, L.J.; Fowler, K.W.; Francisco, M.A.; Reinhold, V.N.; and Wogan, G.N. (1979).
Sterigmatocystin-DNA interactions: identification of a major adduct formed after metabolic activation *in vitro*. Proc. Natl. Acad. Sci. (U.S.A.) 76: 179-183.
- Fahmy, O.G. and Fahmy, M.J. (1970).
Gene elimination in carcinogenesis: reinterpretation of the somatic mutation theory. Cancer Research 30: 195-205.
- Field, C.M.; Devonshire, A.L. and Forde, B.G. (1988).
Molecular evidence that insecticide resistance in peachpotato aphids (*Myzus persicae*, Sulz.) results from amplification of an esterase gene. Biochem. Journal 257:309-312.

- Food and Drug Administration (FDA) (1978).
Assessment of estimated risk resulting from aflatoxins in consumer peanut products and other food commodities, Bureau of Foods, Washington D.C..
- Frei, H, Ciements, J. Howe, D. and Würgler, F.E. (1992).
The genotoxicity of the anti-cancer drug mitoxantrone in somatic and germ cells of *Drosophila melanogaster*. Mutation research, 279: 21-33.
- 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. Mutation Research 203: 297-308.
- Frölich, A. (1989).
Genotoxizitätsprüfung mit *Drosophila melanogaster*: Neue Testerstämme mit erhöhter metabolischer Kapazität, Thesis No. 8850, ETH-Zürich, Zürich, 152pp.
- Frölich, A. and Würgler, F.E. (1989).
New tester strains with improved bioactivation capacity for the *Drosophila* wing spot test. Mutation Research 216: 179-187.
- Frölich, A. and Würgler, F.E. (1990a).
Drosophila wing spot test: Improved detectability of genotoxicity of polycyclic aromatic hydrocarbons. Mutation Research 234: 71-80.
- Frölich, A. and Würgler, F.E. (1990b).
Genotoxicity of ethyl carbamate in the *Drosophila* wing spot test: dependence on genotype-controlled metabolic capacity. Mutation Research 244: 201-208.
- Frölich, A. and Würgler, F.E. (1991a).
The high bioactivation cross for the SMART assay with the wing. *Drosophila* Information Service 70: 246-247.
- Frölich, A. and Würgler, F.E. (1991b).
Increased P450 content and increased aldrin epoxidase activity in an improved set of tester strains for the *Drosophila* SMART assay with the wing. Mutation Research 252: 194.
- Fujikawa, K.; Ryo, H. and Kondo, S. (1985).
The *Drosophila* reversion assay using the unstable zeste-white somatic eye colour system. In: *Evaluation of Short-term Tests for Carcinogens, Progress in Mutation Research*, Ashby, J.; de Serres, F.J.; Draper, M.; Ishidate Jr, M.; Margolin, B.; Matter, B.E. and Shelby, M.D. (eds), vol. 5. Elsevier Science Publishers, Amsterdam, pp 319-324.

- Garcia-Bellido, A. and Dapena, J. (1974).
Induction, detection, and characterization of cell differentiation mutants in *Drosophila*. *Molec. Gen. Genet.* 128: 117-130.
- Garcia-Bellido, A. and Merriam, J.R. (1971).
Parameters of the wing imaginal disc development of *D. melanogaster*. *Developmental Biology* 24: 61-87.
- Garner, R.C.; Miller, E.C. and Miller, J.A. (1972).
Liver microsomal metabolism of aflatoxin B1 to a reactive derivative toxic to *Salmonella typhimurium* TA1530. *Cancer Research* 32: 2058-2066.
- Gelderblom, W.C.A.; Jaskiewicz, K.; Marasas, W.F.O.; Thiel, P.G.; Horak, R.M.; Vleggaar, R. and Kriek, N.P.J. (1988).
Fumonisin: Novel Mycotoxins with cancer promoting activity produced by *Fusarium moniliforme*. *Applied Environmental Microbiology* 54: 1806-1811.
- Gocke, E.; Eckhardt, K.; King, M.-T. and Wild, D. (1982).
Some statistical aspects of spontaneous sex-linked recessive lethal mutations in *Drosophila*. *Mutation Research* 104: 239-242.
- Graf, U. (1972).
Spontaneous Mutation in *Drosophila melanogaster*. *Humangenetik* 16: 27-32.
- Graf, U.; Frei, H.; Kägi, A.; Katz, A.J. and Würzler, F.E. (1989).
Thirty compounds tested in the *Drosophila* wing spot test. *Mutation Research* 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*. *Environmental and Molecular Mutagenesis* 16: 225-237.
- Graf, U.; Heo, O.S. and Olvera Ramirez, O. (1992a).
The genotoxicity of chromium (VI) oxide in the wing spot test of *Drosophila melanogaster* is over 90% due to mitotic recombination. *Mutation Research* 266: 197-203.

- Graf, U.; Juon, H.; Katz, A.J.; Frei, H.J. and Würgler, F.E. (1983).
A pilot study on a new *Drosophila* spot test. *Mutation Research* 120: 233-239.
- Graf, U. and van Schaik, N. (1992).
Improved high bioactivation cross for the wing somatic mutation and recombination test in *Drosophila melanogaster*. *Mutation Research* 271: 59-67.
- Graf, U.; van Schaik, N and Pacella, R.E. (1991).
Improved high bioactivation cross for the SMART wing assay. *Drosophila* Information Service 70: 247-248.
- Graf, U.; van Schaik, N. and Würgler, F.E. (1992b).
Drosophila Genetics a Practical Course. Springer Verlag, Heidelberg, chapter 1, General, pp3-4 and chapter 7, Cytology and Cytogenetics, pp 179-184.
- Graf, U.; Wild, D. and Würgler, F. (1992c).
Genotoxicity of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and related compounds in *Drosophila*. *Mutagenesis* 7: 145-149.
- Graf, U. and Würgler, F.E. (1986).
The present status of validation of the wing spot test in *Drosophila*. In: *Genetic Toxicology of Environmental Chemicals*, Rarnel, C.; Lambert, B. and Magnusson, J. (eds), Part B: Genetic Effects and Applied Mutagenesis, Alan R. Liss, New York, pp 391-398.
- Graf, U. and Würgler, F.E. (1988).
The sex-linked recessive lethal assay and somatic mutation and recombination test in *Drosophila melanogaster*. In: *Evaluation of Short-term Tests for Carcinogens*, Ashby, J.; de Serres, F.J.; Shelby, B.H.; Margolin, M.D.; Ishidate, G.C. and Becking Jr, M., (eds), International Programme on Chemical Safety's Collaborative Study on *in vivo* assays, 2, Cambridge University Press/WHO, Cambridge, pp 2.301-2.309.
- Graf, U.; Würgler, F.E.; Katz, A.J.; Frei, H.J.; Juon, H.; Hall, C.B. and Kale, P.G. (1984).
Somatic Mutation and Recombination Test in *Drosophila melanogaster*. *Environmental Mutagenesis* 6: 153-188.
- Guenguerich, F.D. (1991).
Reactions and Significance of cytochrome P450 enzymes. *The Journal of Biological Chemistry* 266: 10019-10022.

- Haenszel, W. and Kurihara, M. (1968).
Studies of Japanese migrants I, Mortality from cancer and other diseases among Japanese in the U.S.A.. J. Natl. Cancer Inst. 40: 43-68.
- Hällström, I. (1985).
Genetic regulation of the cytochrome P450 system in *D. melanogaster*, I Chromosomal determination of some cytochrome P450-dependent reactions. Chem.-Biol. Interact. 56: 157-171.
- Hällström, I. (1986).
Genetic regulation of the cytochrome P450 system in *Drosophila melanogaster*. In: *Genetic Toxicology of Environmental Chemicals Part B: Genetic Effects and Applied Mutagenesis*, Ramel, C.; Lambert, B. and Magnusson, J. (eds), Alan R. Liss, Inc., New York, pp 419-425.
- Hällström, I. and Blanck, A. (1985).
Genetic regulation of the cytochrome P450 system in *D. melanogaster*, II Localization of some genes regulating cytochrome P450 activity. Chem.-Biol. Interact. 56: 173-184.
- Hällström, I.; Blanck, A. and Atuma, S. (1983).
Comparison of cytochrome P450-dependent metabolism in different developmental stages of *Drosophila melanogaster*. Chem.-Biol. Interactions 46: 39-54.
- Hällström, I.; Blanck, A. and Atuma, S. (1984).
Genetic variation in cytochrome P450 and xenobiotic metabolism in *Drosophila melanogaster*. Biochem. Pharmacol. 33: 13-20.
- Hällström, I. and Gräfsström, R. (1981).
The metabolism of drugs and carcinogens in isolated subcellular fractions of *Drosophila melanogaster*, II, Enzyme induction and metabolism of benzo (a) pyrene. Chem.-Biol. Interactions 34: 145-159.
- Hällström, I; Magnusson, J. and Ramel, C. (1982).
Relation between the somatic toxicity of dimethyl nitrosamine and a genetically determined variation in the level and induction cytochrome P450 in *D. melanogaster*. Mutation Research 92: 161-168.
- Halver, J.E. (1969).
Aflatoxicosis and trout hepatoma. In: *Aflatoxin, Scientific Background, Control and Implications*, Goldblatt, L.A. (ed.), Academic Press, New York, pp 265-306.
- Hartman, P.E.; Ames, B.N.; Roth, J.R.; Barnes, W.M. and Levin, D.E. (1986).
Target sequences for mutagenesis in *Salmonella* histidine-requiring mutants. Environmental Mutagenesis 8: 631-641.

- Hartwig, J.H. and Stossel, T.P. (1979).
Cytochalasin B and the structure of actin gels. *J. Mol. Biol.* 134: 538-553.
- Hay, A. (1988).
How to identify a carcinogen. *Nature* 332: 782-783.
- Hayes, M.A.; Hood, R.D. and Lee, H.L. (1974).
Teratogenic effects of ochratoxin A in mice, *Teratology* 9: 93-97.
- Hirono, I. (Ed.) (1987).
Naturally occurring carcinogens of plant origin: Toxicology, Pathology and Biochemistry, *Bioactive Molecules*, Kodansha/Elsevier, Tokyo, Amsterdam, vol 2.
- Hsieh, D.P. (1987).
Mode of action of mycotoxins. In: *Mycotoxins in Food*, Food Science and Technology- A series of monographs, chapter 7, Krogh, P. (Ed.), Academic Press, New York, pp 149-176.
- Hsieh, D.P.H.; Lin, M.T. and Yao, R.C. (1973).
Conversion of sterigmatocystin to aflatoxin B1. *Biochem. Biophys. Res. Comm.* 52: 992-997.
- Hutchinson, R.D. and Steyn, P.S. (1971).
The isolation and structure of 4-hydroxyochratoxin A and 7-carboxy-3,4-dihydro-8-hydroxy-3-methyl isocoumarin from *Penicillium viridicatum*. *Tetrahedron Letters* 43: 4033-4036.
- Hyrien, O and Buttin, G. (1986).
Gene amplification in pesticide resistant insects. *Trends in Genetics* 2: 275-276.
- IARC monographs (1975).
on the evaluation of the carcinogenic risk of chemicals to man, some naturally occurring substances, Aflatoxins, vol 10, Lyon, France. International Agency for Research on Cancer (IARC)/World Health Organization (W.H.O.), pp 51-72.
- IARC monographs (1987).
on the evaluation of the carcinogenic risk of chemicals to humans, supplement 6, Lyon, France. International Agency for Research on Cancer (IARC)/ World Health Organization (W.H.O.), pp 40-56.
- IARC monographs (1987).
on the evaluation of carcinogenic risk of chemicals to humans, Aflatoxin Group 1, supplement 7, Lyon, France. International Agency for Research on Cancer (IARC)/ World Health Organization (W.H.O.), pp 83-87.

- Ishidate, M. Jr; Harnois, M.C. and Sofuni, T. (1988).
A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutation Research* 195: 151-213
- Ishii, K.; Maeda, K.; Kamataki, T. and Kato, R. (1986).
Mutagenic activation of aflatoxin B1 by several forms of purified cytochrome P450. *Mutation Research* 174: 85-88.
- Jasciewicz, K.; van Rensburg, S.J.; Marasas, W.F.O. and Gelderblom, W.C.A. (1987).
Carcinogenicity of *Fusarium moniliforme* culture material in rats. *JNCI* 78: 321-325.
- Jowett, T.; Farid-Wajidi, M.F.; Oxtoby, E. and Wolf, C.R. (1991).
Mammalian genes expressed in *Drosophila*: a transgenic model for the study of mechanisms of chemical mutagenesis and metabolism, *Embo J.* 10: 1075-1081.
- Kanizawa, M. (1984).
Synergistic effect of citrinine on hepatorenal carcinogenesis of ochratoxin A in mice. In: *Toxic Fungi, their Toxins and Health Hazard*, Kurata, H. and Ueno, Y. (eds), Elsevier/North Holland, Amsterdam, pp 245-254.
- Kanisawa, M. and Suzuki, S. (1978).
Induction of renal and hepatic tumours in mice by ochratoxin A, a mycotoxin. *Gann* 69: 599-600.
- Kastenbaum, M.A. and Bowman, K.O. (1970).
Tables for determining the statistical significance of mutation frequencies. *Mutation Research* 9: 527-549.
- Katz, A.J. (1985).
Genotoxic effects of some base analogs in the *Drosophila* wing spot assay. Abstract, 4th International Conference on Environmental Mutagens, Stockholm, June 24-28, 1985.
- Kikkawa, H. (1958).
Genetic analyses of the resistance to parathion in *Drosophila melanogaster*. *Proc. 10th Intern. Cong. of Genet.* 2: 145.
- Kikkawa, H. (1961).
Genetical studies on the resistance of parathion in *D. melanogaster*. I Gene analyses, *Annu. Rep. Sci. Works Fac. Sci. Osaka Univ.* 9:1-20.
- Konrad, I and Röschenhaler, R. (1977).
Inhibition phenylalanine tRNA synthetase from *Bacillus subtilis* by ochratoxin A. *FEBS Letters* 83: 341-347.

- Krogh, P. (1977).
Ochratoxins. In: *Mycotoxins in Human and Animal Health*, Rodricks, J.V.; Hesseltine, C.W. and Mehلمان, M.A. (eds), Pathox Publishers, Inc., Park Forest South, Illinois, pp489-498.
- Krogh, P. (1987).
Ochratoxins in food. In: *Mycotoxins in Food*, Food Science and Technology- A series of monographs, chapter 5, Krogh, P. (ed.), Academic Press, New York, pp 97-121.
- Krogh, P.; Hald, B.; Piestina, R. and Ceovic, S. (1977).
Balkan (endemic) nephropathy and food borne ochratoxin: Preliminary results of a survey of foodstuffs. *Acta Path. Microbiol. Scand. Sect. B.* 85: 238-240.
- Kuczuc, M. H.; Benson, P.M.; Heath, H. and Hayes, A.W. (1978).
Evaluation of the mutagenic potential of mycotoxins using *Salmonella typhimurium* and *Saccharomyces cerevisiae*. *Mutation Research* 53: 11-20.
- Kurtzman, C.P.; Horn, B.W. and Hesseltine, C.W. (1987).
Aspergillus nomius, a new aflatoxin-producing species related to *A. flavus* and *A. tamarii*. *Anton von Leeuwenhoek* 53: 147-158.
- Lamb, M.J. and Lilly, L.J. (1971).
Induction of recessive lethals in *D.melanogaster* by aflatoxin B1. *Mutation Research* 11: 430-433.
- Lin, J.J.; Liu, C. and Svoboda, D.J. (1974).
Long-term effects of aflatoxin B1 and viral hepatitis on marmoset liver. *Lab. invest.* 30: 267-278.
- Lindsley, M.J. and Grell, E.H. (1968).
Genetic variations of *D.melanogaster*, Carnegie Inst. Wash. Publ. 627, Washington D.C..
- Lindsley, D.L. and Zimm, G. (1985).
The genome of *D.melanogaster*, part 1: Genes A-K. *Drosophila Information Service* 62.
- Linsell, A. and Peers, F. (1977).
Field studies on liver cell cancer. In: *Origins of Human Cancer*, Hiatt, H.H.; Watson, J.D. and Winsten, J.A. (Eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp 549-556.

- Linstrom, H. (1984).
Abstracts of papers, International Chemical Congress of Pacific Basin Societies, Honolulu, Dec. 1984.
- Lovelace, C.E. and Nyathi, C.B. (1977).
Estimation of the fungal toxin zearalenone and aflatoxin contaminating opaque beer in Zambia. *J. Food Agric.* 28: 288-292.
- Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951).
Protein Measurement with Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.
- MacClean-Fletcher, S. and Pollard, T.D. (1980).
Mechanism of action of cytochalasin B on actin. *Cell* 20: 324-341.
- Magnusson, J.; Hällström, I. and Ramel, C. (1978).
Studies on the metabolic activation of vinyl chloride in *D. melanogaster* after pre-treatment with phenobarbital and polychlorinated biphenyls. *Chem.-Biol. Interact.* 24: 287-298.
- Magnusson, J. and Ramel, C. (1990).
Inhibitor of poly (ADP-ribose) transferase potentiates the recombinogenic but not the mutagenic action of alkylating agents in somatic cells *in vivo* in *Drosophila melanogaster*. *Mutagenesis* 5: 511-514
- Mannon, J and Johnson, E. (1985).
Fungi down on the farm. *New Scientist* 105: 12-16.
- Marasas, W.F.O.; Kellerman, T.S.; Gelderblom, W.C.A.; Coetzer, J.A.W.; Thiel, P.G. and van der Lugt, J.J. (1988).
Leukoencephalomalacia in horse induced by fumonisin B1, isolated from *Fusarium moniliforme*. *Onderstepoort J. Vet. Res.* 55:197-203.
- Marasas, W.F.O.; Kellerman, T.S.; Pienaar, J.G. and Naude, T.W. (1976).
Leukoencephalomalacia: a mycotoxicosis of *Equidae* caused by *Fusarium moniliforme* Sheldon. *Onderstepoort J. Vet. Res.* 43: 113-122.
- Marasas, W.F.O.; Kriek, N.P.J.; Fincham, J.E. and van Rensburg, S.J. (1984).
Primary liver cancer and oesophageal basal cell hyperplasia in rats caused by *Fusarium moniliforme*. *Int. J. Cancer* 34: 383-387.
- Marasas, W.F.O.; Kriek, N.P.; van Rensburg, S.J.; Steyn, M. and Schalkwyk, B. (1977).
Occurrence of zearalenone and deoxynivalenol mycotoxins produced by *Fusarium graminearum* in maize in Southern Africa. *S. Afr. J. Sci.* 73: 346-349.

- Maron, D.M. and Ames, B.N. (1983).
Revised methods for the *Salmonella* mutagenicity test. *Mutation Research* 113: 173-215.
- Martin, P.M.D. and Keen, P. (1978).
The occurrence of zearalenone in raw and fermented products from Swaziland and Lesotho. *Sabouraudia* 16: 109-114.
- Marx, J. (1990).
Animal carcinogen testing challenged. *Science* 250: 743-745.
- McCann, J.; Choi, E.; Yamasaki, E. and Ames, B.N. (1975a).
Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals. *Proc. Natl. Acad. Sci. (U.S.A.)* 72: 5135-5139.
- McCann, J.; Spingarn, N.E.; Kabori, J. and Ames, B.N. (1975b).
Detection of carcinogens as mutagens: bacterial tester strains with R-factor plasmids. *Proc. Natl. Acad. Sci. (U.S.A.)* 72:979-983.
- McClintock, B (1929).
A method for making aceto carmine smears permanent. *Stain Technology* 4: 53-56.
- McConnell, E.E. (1987).
Chemical Carcinogenesis. *Science* 238: 259.
- McGrath, J. and Solter, D. (1984).
Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* 37: 179-183.
- McGregor, H.C. (1983).
Working with Animal Chromosomes, second edition. John Wiley and Sons, Chichester.
- Merriam, J.R. and Garcia-Bellido, A. (1972).
A model for somatic pairing derived from somatic crossing over with the third chromosome rearrangements in *Drosophila*. *Mol. Gen. Genet.* 115: 302-313.
- Metz, J.; Livni, N.; Simon, I.W.; Uys, C.J.; Anderson, J.D. and Isaacson, M. (1986).
Cancer Registry of South Africa, Annual Report, S.A.I.M.R.
- Metz, J.; Livni, N.; Simon, I.W.; Uys, C.J.; Anderson, J.D. and Isaacson, M. (1987).
Cancer Registry of South Africa, Annual Report, S.A.I.M.R.

- Metzler, D.E. (1977).
Oxygen and Generation of ATP, *Biochemistry, the Chemical Reactions of Living Cells*, Chapter 10, Academic Press, New York, pp 568-571.
- Mollet, P. and Würigler, F.E. (1974).
Detection of somatic recombination and mutation in *Drosophila*: a method for testing genetic activity of chemical compounds. *Mutation Research* 25: 421-424.
- Mori, H.; Sugie, S.; Yoshimi, N.; Kitamura, J.; Niwa, M.; Hamasaki, T. and Kawai, K. (1986).
Genotoxic effects of a variety of sterigmatocystin-related compounds in the hepatocyte/DNA-repair test and the *Salmonella* microsome assay. *Mutation Research* 173: 217-222.
- Mouchès, C., Pasteur, N.; Bergé, J.B.; Hyrien, O.; Raymond, M.; de Saint Vincent, B.R.; de Silvestri, M. and Giorghiou, G.P. (1986).
Amplification of an esterase gene is responsible for insecticide resistance in a California *Culex* mosquito. *Science* 233: 778-780.
- Muir, C.; Waterhouse, J.; Mack, T.; Powell, J. and Whelan, S. (1987).
Cancer Incidence in Five Continents, volume V, International Agency for Research on Cancer, Scientific Publications Number 88, Lyon.
- Müller, H.J. (1927).
The problem of genic modification, 5th International Genetics Congress, Berlin, Z. ind. Abst. Vererb. Lehre, I (suppl. 1) 234-260.
- Munoz, N. and Bosch, F.X. (1987).
Epidemiology of hepatocellular carcinoma, In: *Neoplasms of the Liver*, Okuda, K. and Ishak, K.G. (eds), Springer, Tokyo, pp 3-19.
- Nagao, M. Wakabayashi, K. and Sugimura, T. (1985).
Mutagens in food and drinks and their carcinogenicity. In: *Mutagenicity Testing in Environmental Pollution Control*, Zimmerman, F.K. and Taylor-Mayer, R.E. (eds), chapter 5, Ellis Horwood Limited Publishers, Chichester, pp 69-85.
- Nebert, D.W. and Jensen, N.M. (1979).
The Ah locus: genetic regulation of the metabolism of carcinogens, drugs and other environmental chemicals. *Crit. Rev. Biochem.* 6: 401.
- Newberne, P.M. and Wogan, G.N. (1968).
Sequential morphological changes in aflatoxin B1 carcinogenesis in the rat. *Cancer Research* 28: 770-781.
- NIH guidelines for the laboratory use of chemical carcinogens, NIH publication N° 81-2385, May 1981, Public Health Service, National Institute of

Health, United States Department of Health and Human Services, pp 1-10.

Nöthiger, R. (1970).

Sucrose density separation: a method for collecting large numbers of *Drosophila* larvae. *Drosophila* Information Service 45: 177.

Nyathi, C.B.; Dube, N. and Hasler, J. (1991).

Mycotoxins. African Newsletter on Occupational Health and Safety: Proceedings of an International Course on Detection of Health Hazards in Human Populations exposed to chemical mutagens and carcinogens, Harare, Zimbabwe 9-20 September 1991, edited by Lehtinen, S. and Salmenius, R., pp 18-30.

Nyathi, C.B.; Mutiro, C.F.; Hasler, J.A. and Chetsanga, C.J. (1987).

A survey of urinary aflatoxin in Zimbabwe. *Int. J. Epidemiology* 16: 516-519.

Okuda, K. and Beasley, R.P. (1982).

Epidemiology of hepatocellular carcinoma. In: *Hepatocellular carcinoma*, Okuda, K. and MacKay, J. (eds), VICC Report 17, Geneva, Switzerland, pp 34-47.

Omura, T and Sato, R. (1964a).

The carbon monoxide-binding pigment of liver microsomes I Evidence for its haemoprotein nature, *Journal of Biological Chemistry* 239: 2370-2378.

Omura, T and Sato, R. (1964b).

The carbon monoxide-binding pigment of liver microsomes II Solubilization, purification and properties. *The Journal of Biological Chemistry* 239: 2379.

Ong, C. (1971).

Mutagenic activities of aflatoxin B1 and G1 in *Neurospora crassa* Molecular. Gen. Genetics 111: 159-170.

Oppenorth, F.J. (1984).

Biochemistry of insecticide resistance. *Pestic. Biochem. Physiol.* 22: 187.

Parkin, D.M.; Stjernsward, J. and Muir, C.S. (1984).

Estimates of the world wide frequency of twelve major cancers, *Bull. W.H.O.* 62:163-182.

Paro, R. (1990).

Imprinting a determined state into the chromatin of *Drosophila*. *Trends in Genetics* 6:416-421.

- Peers, F.; Bosch, X.; Kaidor, J.; Linsell, A. and Pluijman, M. (1987).
Aflatoxin exposure, hepatitis B virus infection and liver cancer in Swaziland, Intl. J. Cancer 39: 545-553.
- Peers, F.G. and Linsell, C.A. (1973).
Dietary aflatoxins and liver cancer-a population based study in Kenya. British J. Cancer 27: 473-484.
- Plapp, F.W. (1984).
The genetic basis of insecticide resistance in the housefly: evidence that a single locus plays a major role in metabolic resistance to insecticides. Pesticide Biochem. Physiol. 22:194.
- Pohland, A.E. and Wood, G.E. (1987).
Occurrence of mycotoxins in food. In: *Mycotoxins in Food*, Krogh, P. (Ed.), chapter 3, Academic Press, London, pp 35-64.
- Postlethwait, J.H. (1978).
Clonal analysis of *Drosophila* cuticular patterns. In: *The Genetics and Biology of Drosophila*, Ashburner, M. and Wright, T.R.F. (eds), The Genetics and Biology of *Drosophila*, vol. 2c, Academic Press, London, pp 359-441.
- Purchase, I.F.H.; Lonstaff, E.; Ashby, J.; Styles, J.A.; Anderson, D.; Lefevre, P.A. and Westwood, F.R. (1976).
Evaluation of six short-term tests for detecting chemical carcinogens and recommendations for their use. Nature (London) 264: 624-629.
- Purchase, I.F.H. (1981).
Summary report on the performance of *in vivo* assays, Evaluation of Short-term Tests for Carcinogens, Report of the International Collaborative Program. In: *Progress in Mutation Research*, de Serres, F.J. and Ashby, J. (eds), vol. 1, Elsevier/ North-Holland, New York, pp 88-95.
- Purchase, I.F.H. and van der Watt, J.J. (1970).
Carcinogenicity of sterigmatocystin. Fd Cosmet. Toxicol. 8: 289-295.
- Purdom, C.E., Dyer, K.F. and Papworth, D.G. (1968).
Spontaneous mutation in *Drosophila*: studies on the rate of mutation in mature and immature male germ cells. Mutation Research 5: 133-146.
- Raina, V.; Williams, C.J. and Gurtoo, H.L. (1983).
Genetic expression of aflatoxin metabolism. Effects of 3-methylcholanthrene and β -naphthoflavone on hepatic microsomal metabolism and mutagenic activation of aflatoxins. Biochemical Pharmacology 32: 3755-3763.

- Razin, A. and Riggs, A.D. (1980).
DNA methylation and gene function. *Science* 210:604-610.
- Reddy, J.K. and Svoboda, D.J. (1975).
AFB1-induced liver tumours in *Tupaia glis* (tree shrews), a non-human primate. *Fed. Proc.* 34: 827.
- Reference maps of the salivary gland chromosomes of *Drosophila melanogaster*, Supplement to *Journal of Heredity* vol. 26 (1935).
- Reference maps of salivary gland 2R chromosome of *Drosophila melanogaster*, Supplement to *Journal of Heredity* vol. 30 (1939).
- Sato, S.; Matsushima, T.; Tanaka, N.; Sugimura, T. and Takashima, F. (1973).
Hepatic tumours in the guppy (*Lebistes reticulatus*) induced by aflatoxin B1, dimethylnitrosamine and 2-acetylaminofluorene. *J. Natl. Acad. Inst.* 50: 765-778.
- Schuller, P.L.; Stoloff, L. and Van Egmond, H.P. (1982).
Limits and regulation. In: *Environmental Carcinogens- selected methods of analysis*, vol. 5, *Some Mycotoxins*, H. Egan, Stoloff, L., Castegnaro, M.; Scott, P.; O'Neill, I.K. and Bartsch, H. (eds), International Agency for Research on Cancer, France, pp 107-116.
- Scott, P.M. (1985).
Aflatoxins, pp 22-24. In: Scott, P.M.; Trenholm, H.L. and Sutton, M.D. (eds). *Mycotoxins, a Canadian Perspective*. NRCC Publication number 22848, National Research Council of Canada, Ottawa.
- Scott, P.; Panalaks, P.T.; Kariore, S. and Miles, W.P. (1978).
Determination of zearalenone in cornflakes and other corn-based foods by thin layer chromatography, high pressure liquid chromatography and gas-liquid chromatography. *J. Assoc. Off. Anal. Chem.* 61: 593-600.
- Selby, P.B. and Olson, W.H. (1981).
Methods and criteria for deciding whether specific locus mutation rate data in mice indicates a positive, negative or inconclusive result. *Mutation Research* 83: 403-418.
- Sekita, S.; Yoshihira, K. and Natori, S. (1977).
Chaetoglobosins G and J, cytotoxic indol-3-yl [13] cytochalasins from *Chaetomium globosum*. *Tetrahedron Letters* pp 2771-2774.

- Shank, R.C.; Gordon, J.E.; Wogan, G.N.; Nondasuta, A. and Subhamani, B. (1972a).
Dietary aflatoxins and human liver cancer. III Field survey of rural Thai families for ingested aflatoxins. *Food. Cosmet. Toxicol.* 10: 71-84.
- Shank, R.C.; Wogan, G.N.; Gibson, J.B. and Nondasuta, A. (1972b).
Dietary aflatoxins and human liver cancer. II Aflatoxins in market foods and foodstuffs in Thailand and Hong Kong. *Food. Cosmet. Toxicol.* 10: 61-69.
- Smith, T.K. and Moss, M.O. (1985).
Mycotoxins: Formation, Analysis and Significance. John Wiley and Sons, New York, 149 pp.
- Sobels, F.H. (1974).
The advantages of *Drosophila* for mutation studies. *Mutation Research* 26: 277-284.
- Spencer, W.P. and Stern, C. (1948).
Experiments to test the validity of the linear R-dose/mutation frequency relationship in *Drosophila* at low dosage. *Genetics* 33: 43-74.
- Stark, A.A. (1980).
Mutagenicity and carcinogenicity of mycotoxins: DNA binding as a possible mode of action. *Ann. Rev. Microbiol.* 34: 235-262.
- Stormer, F.C. and Pedersen, J.I. (1980).
Formation of 4-hydroxyochratoxin A from ochratoxin A by rat liver microsomes. *Appl. Environ. Microbiol.* 39: 971-975.
- Stormer, F.C.; Hansen, C.E.; Pedersen, J.I.; Huistendahl, G. and Aasen, A.J. (1981).
Formation of (4R)- and (4s)-4-hydroxyochratoxin A from ochratoxin A by liver microsomes from various species. *Appl. Environ. Microbiol.* 42: 1051-1056.
- Sugimura, T. (1978).
Let's be scientific about the problem of mutagens in cooked food. *Mutation Research* 55: 149-152.
- Swenson, D.H.; Miller, E.C. and Miller, J.A. (1974).
Aflatoxin B1-2,3-oxide: evidence for its formation in rat liver *in vivo* and by human liver microsomes *in vitro*. *Biochem. Biophys. Res. Commun.* 60: 1036-1043.

- Szabad, J.; Soos, I.; Polgar, P. and Hejja, G. (1983).
Testing the mutagenicity of malondialdehyde and formaldehyde by the *Drosophila* mosaic and the sex-linked recessive lethals test. *Mutation Research* 113: 117-133.
- Szmuness, W. (1978).
Hepatocellular carcinoma and the Hepatitis B virus, evidence for a causal association. *Prog. Med. Virol.* 24: 40-69.
- Tannenbaum, A. (1942).
The genesis of growth of tumours III Effects of a high fat diet. *Cancer Research* 2: 468-493.
- Tates, A.D. (1971).
Cytodifferentiation during spermatogenesis in *Drosophila*, Thesis, University of Leiden. (In Vogel, 1975).
- Tashiro, F.; Kawabata, Y.; Naoi, M. and Ueno, Y. (1980).
In: *Medical Mycology* (Zentra bl. Bakteriöl. Parasiten kd. Infektionskr. Hyg., Suppl. 1, 8, Gustav Fischer Verlag, Stuttgart, p 311.
- Tennant, R.W.; Margolin, B.H.; Shelby, M.D.; Zeiger, E.; Haseman, J.K.; Spalding, J.; Caspary, W.; Resnick, M. Stasiewicz, S.; Anderson, B. and Minor, R. (1987).
Mutagenicity in rodent from *in vitro* genetic toxicity assays. *Science* 236: 933-944.
- Tomatis, L. (1990).
Cancer: Causes, occurrence and control, W.H.O./ IARC scientific publications number 100, Lyon, France, pp 43-51.
- Towbin, H.; Staehelin, T. and Gordon, J. (1979).
Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedure and some applications. *Proceedings of the National Academy of Science (U.S.A.)* 76:4350.
- Tsukamoto, M. (1958).
New Mutants. *Drosophila* Information Service 32:87.
- Turner, W.B. (1971).
Fungal Metabolites, Academic Press, London.
- Ueno, Y. (1984).
The toxicology of mycotoxins. *CRC Crit. Rev. Toxicol.* 14: 99-132.
- Ueno, Y. (1987).
Trichothecenes in food. In: *Mycotoxins in Food*, Food Science and Technology- A series of monographs, chapter 6, Krogh, P. (Ed.), Academic Press, New York, pp 123-147.

- Ueno, Y. and Kubota, K. (1976).
DNA-attacking ability of carcinogenic mycotoxins in recombination-deficient mutant cells of *Bacillus subtilis*. *Cancer Research* 36: 445-451.
- Ueno, Y; Kubota, K.; Ito, T. and Nakamura, I. (1978).
Mutagenicity of carcinogenic mycotoxins in *Salmonella typhimurium*. *Cancer Research* 38: 536.
- Umeda, M; Tsutsui, T. and Saito, M. (1977).
Mutagenicity and inducibility of DNA single-strand breaks and chromosome aberrations by various mycotoxins. *Gann* 68: 619-625.
- Vainio, H. and Wilbourn, J. (1991).
Identification of carcinogens within the IARC monographs programme. African Newsletter on Occupational Health and Safety: Proceedings of an international course on the detection of health hazards in human populations exposed to chemical mutagens and carcinogens, Harare, Zimbabwe, 2-20 September 1991, edited by Lehtinen, S. and Saimenius, R., pp 1B-30.
- Valencia, R.; Abrahamson, S.; Lee, W.R.; von Halle, E.S.; Woodruff, R.C.; Würgler, F.E. and Zimmering, S. (1984)
Chromosome mutation tests for mutagenesis in *Drosophila melanogaster*, a report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutation Research* 134: 61-88.
- van Schaik, N. and Graf, U. (1991).
Genotoxicity evaluation of five tricyclic antidepressants in the wing somatic mutation and recombination test in *Drosophila melanogaster*. *Mutation Research*: 99-104.
- van Rensburg, S.J.; Cook-Mozaffari, P.; van Schalkwyk, D.J.; van der Watt, J.J.; Vincent, T.J. and Purchase, I.F.H. (1985).
Hepatocellular carcinoma and dietary aflatoxin in Mozambique and Transkei. *British J. of Cancer* 51: 713-726.
- van Rensburg, S.J.; van Schalkwyk, G.C. and van Schalkwyk, D.J. (1990).
Primary liver cancer and aflatoxin intake in Transkei. *JEPTO* 10: 11-16.
- Vesonder, R.F. and Horn, B.W. (1985).
Sterigmatocystin in dairy cattle feed contaminated with *Aspergillus versicolor*. *Applied Environ. Microbiology* 49: 234-235.
- Vesselnovitch, S.D.; Mihailovich, N.; Wogan, G.N.; Lombard, L.S. and Rao, K.V.N. (1972).
Aflatoxin B1, a hepatocarcinogen in the infant mouse, *Cancer Research* 32: 2289-2291.

- Vogel, E.W. (1975).
Some aspects of the detection of potential mutagenic agents in *Drosophila*. *Mutation Research* 29: 241-250.
- Vogel, E.W. (1981).
Short-term Tests for Chemical Carcinogens, Stich, H.F. and San, R.H.C. (eds), Springer, New York, pp 379-398.
- Vogel, E.W. (1980).
Genetical relationship between resistance to insecticides and promutagens in two *Drosophila* populations. *Arch. Toxicol.* 43: 201-211.
- Vogel, E.W. (1985).
The *Drosophila* somatic recombination and mutation assay (SRM) using the white-coral somatic eye system. In: *Evaluation of Short-term tests for Carcinogens*, Ashby, J.; de Serres, F.J.; Draper, M.; Ishidate Jr, M.; Margolin, B.; Matter, B.E. and Shelby, M.D. (eds), *Progress in Mutation Research*, vol. 5, Amsterdam: Elsevier Science Publishers, pp 313-317.
- 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).
Comparison of mutation induction in germ cells and somatic tissue in three *Drosophila* assays: the sex-linked recessive lethals test, the somatic mutation/recombination test, and the chromosome loss assay. In: *Evaluation of Short-term Tests for Carcinogens. International Programme on Chemical Safety's Collaborative study on in vivo assays*, Ashby, J.; de Serres, F.J.; Shelby, B.H.; Margolin, M.D.; Ishidate, G.C. and Becking Jr, M., (eds), chapter 2, Cambridge University Press/WHO, Cambridge, pp 2.286-2.297.
- Vogel, E.W.; Bulzhenkov, V.E.; Fujikawa, K.; Graf, U.; Ivanov, V.I.; Kilbey, B.; Würgler, F.E. (1988).
Summary Report on the performance of the *Drosophila* assays. In: *Evaluation of Short-term tests for Carcinogens, International Programme on Chemical Safety's Collaborative Study on in vivo assays*, Ashby, J.; de Serres, F.J.; Shelby, B.H.; Margolin, M.D.; Ishidate, G.C. and Becking Jr, M., (eds), chapter 2, Cambridge University Press/WHO, Cambridge, pp 2.277-2.285.
- Vogel, E.W.; Frei, H.; Fujikawa, K.; Graf, U.; Kondo, S.; Ryo, H. and Würgler, F.E. (1985).
Summary report on the performance of the *Drosophila* assays. In: *Evaluation of Short-term Tests for Carcinogens, Progress in Mutation Research*, vol. 5, Ashby, J.; de Serres, F.J.; Draper, M.; Ishidate Jr, M.; Margolin, B.; Matter, B.E. and Shelby, M.D. (eds), Elsevier Science Publishers, Amsterdam, pp 47-57.

- Vogel, E.W. and Leigh, B. (1975).
Concentration-effect studies with MMS, TEB, 2,4,6-C13-PDMT, and DEN on the induction of dominant and recessive lethals, chromosome loss and translocations in *Drosophila* sperm, *Mutation Research* 29: 383-396.
- Vogel, E.W. and Luërs, H. (1974).
A comparison of adult feeding to injection in *Drosophila melanogaster*. *Drosophila Information Service* 51: 113-114.
- Vogel, E.W. and Sobels, F.H. (1976).
The function of *Drosophila* in genetic toxicology testing In: *Chemical Mutagens, Principles and Methods for their Detection*, Hollaender, A. (ed.), vol. 4, Plenum Press, New York, pp 93-142.
- Vogel, E.W. and Würigler, F.E. (1990).
Proposal to replace OECD guideline 477 "Genetic Toxicology: Sex-linked Recessive Lethals Test in *D.melanogaster*" by a new guideline "Genetic Toxicology: Somatic Mutation and Recombination Test (SMART) in *D. melanogaster*" (unpublished).
- Vogel, E.W.; Zijlstra, J.A. and Blijleven, W.G.H. (1983).
Mutagenic activity of selected aromatic amines and polycyclic hydrocarbons in *Drosophila melanogaster*. *Mutation Research* 107: 53-77.
- Wales, J.H. and Sinnhuber, R.O. (1972).
Hepatomas induced by aflatoxin in the sockeye salmon (*Oncorhynchus nerca*). *J. Nat. Cancer Inst.* 48: 1529-1530.
- Wallace, B. (1970).
Spontaneous mutation rates for sex-linked recessive lethals in the two sexes of *Drosophila melanogaster*, *Genetics* 64: 553-557.
- Watanabe, T.; Taguchi, i.; Kitamura, Y.; Tsuyama, K.; Fugiki, H.; Tanooka, H. and Sugimura, T. (1982).
Induction of histidine decarboxylase activity in mouse skin after application of indole alkaloids, a new class of tumour promoter. *Biochim. Biophys. Res. Commun.* 109: 478-485.
- Waterhouse, J.; Muir, C.; Correa, P. and Powell, J. (1976).
Cancer Incidence in Five Continents, volume III, International Agency for Research on Cancer Scientific Publications Number 15, Lyon.
- Waterhouse, J.; Muir, C.; Shanmugaratnam, K. and Powell, J. (1982).
Cancer Incidence in Five Continents, volume IV, International Agency for Research on Cancer Scientific Publications Number 42, Lyon.

- Vogel, E.W. and Leigh, B. (1975).
Concentration-effect studies with MMS, TEB, 2,4,6-C13-PDMT, and DEN on the induction of dominant and recessive lethals, chromosome loss and translocations in *Drosophila* sperm, *Mutation Research* 29: 383-396.
- Vogel, E.W. and Luërs, H. (1974).
A comparison of adult feeding to injection in *Drosophila melanogaster*. *Drosophila Information Service* 51: 113-114.
- Vogel, E.W. and Sobels, F.H. (1976).
The function of *Drosophila* in genetic toxicology testing. In: *Chemical Mutagens, Principles and Methods for their Detection*, Hollaender, A. (ed.), vol. 4, Plenum Press, New York, pp 93-142.
- Vogel, E.W. and Würgler, F.E. (1990).
Proposal to replace OECD guideline 477 "Genetic Toxicology: Sex-linked Recessive Lethals Test in *D.melanogaster*" by a new guideline "Genetic Toxicology: Somatic Mutation and Recombination Test (SMART) in *D. melanogaster*" (unpublished).
- Vogel, E.W.; Zijlstra, J.A. and Blijleven, W.G.H. (1983).
Mutagenic activity of selected aromatic amines and polycyclic hydrocarbons in *Drosophila melanogaster*. *Mutation Research* 107: 53-77.
- Wales, J.P. and Sinnhuber, R.O. (1972).
Hepatomas induced by aflatoxin in the sockeye salmon (*Oncorhynchus nerca*). *J. Nat. Cancer Inst.* 48: 1529-1530.
- Wallace, B. (1970).
Spontaneous mutation rates for sex-linked recessive lethals in the two sexes of *Drosophila melanogaster*, *Genetics* 64: 553-557.
- Watanabe, T.; Taguchi, I.; Kitamura, Y.; Tsuyama, K.; Fugiki, H.; Tanooka, H. and Sugimura, T. (1982).
Induction of histidine decarboxylase activity in mouse skin after application of indole alkaloids, a new class of tumour promoter. *Biochim. Biophys. Res. Commun.* 109: 478-485.
- Waterhouse, J.; Muir, C.; Correa, P. and Powell, J. (1976).
Cancer Incidence in Five Continents, volume III, International Agency for Research on Cancer Scientific Publications Number 15, Lyon.
- Waterhouse, J.; Muir, C.; Shanmugaratnam, K. and Powell, J. (1982).
Cancer Incidence in Five Continents, volume IV, International Agency for Research on Cancer Scientific Publications Number 42, Lyon.

- Waters, L.C. and Nix, C.E. (1988).
Regulation of insecticide resistance related cytochrome P450 expression in *D. melanogaster*. *Pesticide Biochem. Physiol.* 30: 214-227.
- Waters, L.C.; Nix, C.E. and Epier, J.L. (1983).
Studies on the relationship between dimethyl nitrosamine demethylase activity and dimethylnitrosamine-dependent mutagenesis in *Drosophila*. *Chem.-Biol. Interact.* 46: 55.
- Wehner, F.C.; Marasas, W.F.O. and Thiel, P.G. (1978).
Lack of mutagenicity to *Salmonella typhimurium* of some *Fusarium* mycotoxins. *Applied Environmental Microbiology* 35: 569-662.
- Wei, C. and MacLaughlin, S. (1974).
Structure-function relationship in the 12,13-epoxytrichothecenes, novel inhibitors of protein synthesis. *Biochem. Biophys. Res. Commun.* 57: 838.
- Weinstein, I.B. and Groopman, J.D. (1991).
Environmentally-induced cancers: causes, mechanisms and prevention. *African Newsletter on Occupational Health and Safety: Proceedings of an international course on the detection of health hazards in human populations exposed to chemical mutagens and carcinogens, Harare, Zimbabwe, 2-20 September 1991, edited by Lehtinen, S. and Salminen, R., pp 32.*
- Wild, C.P.; Jiang, Y.Z.; Sabbioni, G.; Chapot, B. and Montesano, R. (1990).
Evaluation of methods for the quantitation of AF-albumin adducts and their application to human exposure assessment. *Cancer Res.* 50: 245-257.
- Wild, C.P.; Pionneau, F.A.; Montesano, R.; Mutiro, C.F. and Chetsanga, C.J. (1987).
Aflatoxin detected in human breast milk by immunoassay. *Int. J. Cancer* 40: 328-333.
- Wilkinson, C.F. and Brattstan, L.B. (1972).
Microsomal drug metabolizing enzymes in insects, *Drug Metabol. Rev.* vol 1, n° 2, pp 153-228.
- Willett, W.C.; Stampfer, M.J.; Colditz, G.A.; Rosner, B.A.; Speizer, F.E. (1990).
Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women, *New England J. of Med.* 323: 1664-1672.

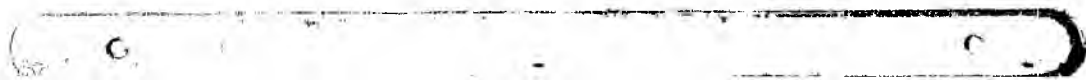
- Williams, B.J. and Wilson, E. (1976).
Principles and Techniques of Practical Biochemistry, Edward Arnold Publishers, London, p 256.
- Wogan, G.N. (1969).
Naturally occurring carcinogens in foods. *Prog. Tumour Res. (Basel)* 11: 134-162.
- Wogan, G.N. (1973).
Aflatoxin carcinogenesis, *Methods in Cancer Research* 7: 309.
- Wogan, G.N. and Newberne, P.M. (1967).
Dose-response characteristics of AFB1 carcinogenesis in the rat. *Cancer Research* 27: 2370-2376.
- Wogan, G.N.; Edwards, G.S. and Newberne, P.M. (1971).
Structure-activity relationship in toxicity and carcinogenicity of aflatoxins and analogs. *Cancer Research* 31: 1936-1942.
- Wolf, C.R. (1986).
Cytochrome P450's: a multigene family involved in carcinogen metabolism. *Trends in Genetics* 2: 209-214.
- Wolf, C.R. (1990).
Metabolic factors in cancer susceptibility. *Cancer Surveys* 9: 437-474.
- Wong, J.J. and Hsieh, D.P.H. (1976).
Mutagenicity of aflatoxins related to their metabolism and carcinogenic potential. *Proc. Natl. Acad. Sci. (U.S.A.)* 73: 2241-2244.
- Wong, J.J.; Singh, R. and Hsieh, D.P.H. (1977).
Mutagenicity of fungal metabolites related to aflatoxin biosynthesis. *Mutation Research* 44: 447-450.
- Woodruff, R.C.; Slatko, B.E. and Thompson, J.N. (Jr) (1983).
Factors affecting mutation rates in natural populations. In: *The Genetics and Biology of Drosophila*, Ashburner, M. (ed.), vol. 3c, Academic Press, London, pp 3-124.
- Würgler, F.E. (1983).
The sex-linked recessive lethal assay with *Drosophila melanogaster*. *Trends in Pharmacological Sciences* 4: 492-494.
- Würgler, F.E.; Friederich, U. and Schlatter, J. (1991).
Lack of Mutagenicity of ochratoxin A and B, citrinin, patulin and cneistine in *Salmonella typhimurium* TA102. *Mutation Research* 261: 209-216.

- Würgler, F.E. and Graf, U. (1985).
Mutagenicity testing with *Drosophila melanogaster*. In: *Basic Applied Mutagenesis, with Special Reference to Agricultural Methods in Developing Countries*, Muhammed, A. and von Borstel, R.C. (eds), Plenum Press, New York, pp 343-372.
- Würgler, F.E. and Graf, U. (1990).
Genotoxicity assays with somatic cells of *Drosophila melanogaster*. *Rev. Latinoamer. Genet.* vol extraord., 181-196.
- Würgler, F.E., Graf, U. and Berchtold, W. (1975).
Statistical problems connected with the sex-linked recessive lethal test in *Drosophila melanogaster*! The use of the Kastenbaum-Bowman test. *Archiv für Genetik* 48:158-178.
- Würgler, F.E.; Graf, U. and Frei, H. (1985).
Somatic mutation and recombination in wings of *Drosophila melanogaster*. In: *Collaborative Studies of Short-term tests for Carcinogens*, Progress in Mutation Research 5, Elsevier/North Holland, Amsterdam, pp 325-340.
- Würgler, F.E.; Graf, U.; Frei, H. and Juon, H. (1983).
Genotoxic activity of the anticancer drug methotrexate in somatic cells of *Drosophila melanogaster*. *Mutation Research* 122: 321-328.
- Würgler, F.E.; Graf, U.; Frei, H. and Juon, H. (1984).
Detection of point mutations, chromosome aberrations and somatic recombination in *Drosophila melanogaster*. In: *Critical Evaluation of Mutagenicity Tests*, Bass, R.; Glocklin, V.; Grosdanoff, P. and Henschler, D. (eds), BGA Schriften, MMV Medizin, Munich, pp 185-223.
- Würgler, F.E., Sobels, F.H. and Vogel, E. (1977).
Drosophila as assay system for detecting mutagenic changes. In: *Handbook of Mutagenicity Testing*, Kilby, B.J.; Legator, M.; Nichols, W. and Ramel, C. (eds), Elsevier/North-Holland Biomedical Press, pp 335-373.
- Würgler, F.E., Sobels, F.H. and Vogel, E. (1984).
Drosophila as an assay system for detecting genetic changes. In: *Handbook of Mutagenicity Test Procedures*, Kilby, B.J.; Legator, M.; Nichols, W. and Ramel, C. (eds), Second Edition, Elsevier Science Publishers, Amsterdam, pp 555-601.
- Würgler, F.E. and Vogel, E.W. (1985).
In vivo mutagenicity testing using somatic cells of *D. melanogaster*. In: *Chemical Mutagens, Principles and Methods for their Detection*, de Serres, F.J. (ed.), vol. 10, Plenum Press, New York, pp 1-72.

- Yahara, I.; Harada, F.; Sekita, S.; Yoshihira, K. and Natori, S. (1982).
Correlation between the effects of 24 different cytochalasins on cellular structures and cellular events and those on actin *in vitro*. J. Cell Biol. 92: 69-78.
- 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.
- Younes, M.; Siegers, C.P.; Filser, J.G. (1979).
Effect of dithiocarb and dimethyl sulphoxide on irreversible binding of C14-bromobenzene to rat liver microsomal protein. Archives of Toxicology 42: 289-293.
- Zeiger, E. (1987).
Carcinogenicity of mutagens: Predictive capability of the *Salmonella* mutagenesis assay for rodent carcinogenicity. Cancer Research 47: 1287-1296.
- Zimmerman, F.K. (1985).
Introduction. In: *Mutagenicity Testing in Environmental Pollution Control*, Zimmerman, F.K. and Taylor-Mayer, R.E. (eds), Ellis Horwood Limited Publishers, Chichester, pp 11-13.
- Zimmerman, F.K. and Taylor-Mayer, R.E. (1985).
Mutagenicity testing- some background information. In: *Mutagenicity Testing in Environmental Pollution Control*, Zimmerman, F.K. and Taylor-Mayer, R.E. (Eds), chapter 3, Ellis Horwood Limited Publishers, Chichester, pp 23-46.
- Zijlstra, J.A.; Vogel, E.W. and Breimer, D.D. (1984).
Strain differences and inducibility of microsomal oxidative enzymes in *D.melanogaster* flies, Chem.-Biol. Interact. 48: 317-338.
- Zijlstra, J.A.; Vogel, E.W. and Breimer, D.D. (1987).
Pharmacological and toxicological aspects of mutagenicity research in *Drosophila melanogaster*. In: *Reviews in Biochemical Toxicology*, Hogson, E.; Bend, J.R. and Philpot, R.M. (eds), chapter 8, Elsevier, Amsterdam, pp 121-154.

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Author: Pacella Rosana Elizabeth.

Name of thesis: Genotoxicity of mycotoxins in an improved Drosophila wing spot test and other short-term effects.

PUBLISHER:

University of the Witwatersrand, Johannesburg

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