

The HET-MN Assay: An Alternative Approach for Basic and Follow-up Genotoxicity Testing - Further Results.

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Introduction

Need for a new alternative genotoxicity assay:

- current in vitro genotoxicity assays are sensitive but a considerable part of in vitro positives may not be biological relevant. => in vivo follow up testing is often necessary which is most often a micronucleus test (MNT)
- the new chemical legislation REACH: toxicological hazard identification for numerous „existing substances“
- 7th amendment of cosmetics directive 76/768/EEC: As from 2009 prohibition of animal testing and marketing ban for cosmetic products for which cosmetic ingredients (or the cosmetic products) were tested on animals

Possible solution:

- an alternative method for the micronucleus test (MNT) in vivo has to consider a more realistic situation of the toxicokinetics and -dynamics of a test substance in mammals than is expressed at present in in vitro assays
- the hen's egg represents an approach to this demand ^{1,2,3}

Results

Substance	In vivo MNT	Comment / Ref.	HET-MN	Ref.
2-Acetylaminofluorene	Positive	A pro-mutagen CSGMT, 1992	Positive	Submitted to Mutation Research
Acrylamide	Positive	A pro-mutagen Shelby and Witt, 1995	Positive	Submitted to Mutation Research
Azorubin (E-122)	Negative	Kim and Margolin, 1999	Negative	Submitted to Mutation Research
Cadmium chloride	Positive	Fahmy and Aly, 2000	Positive	Submitted to Mutation Research
Cyclophosphamide	Positive	A pro-mutagen CSGMT, 1992	Positive	Wolf et al., 2002
Cytarabine	Positive	Mavournin et al., 1990	Positive	Submitted to Mutation Research
Dimethylbenz[<i>a</i>]anthracene	Positive	A pro-mutagen Shelby and Witt, 1995	Positive	Wolf et al., 2002
Dipotassium monochromate	Positive	Heddle et al., 1983 CSGMT, 1992	Positive	Submitted to Mutation Research
Epirubicine	not available	Anti-cancer drug, intercal.	Positive	Preliminary results

Methods

Egg incubation, substance administration, blood sampling, staining of blood smears, scoring of micronuclei were done according to Wolf et al. ^{1,2,3}. The test procedure is shown in figure 1.

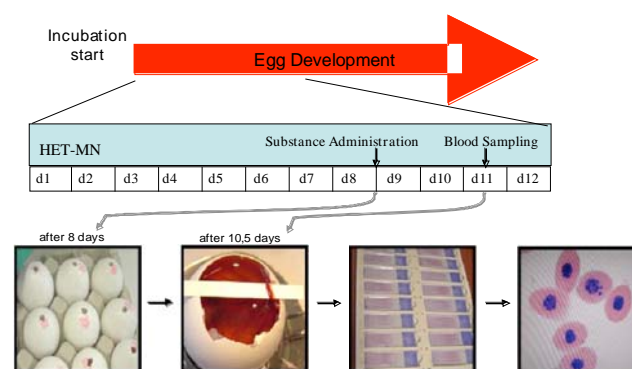


Figure 1: Test procedure of the HET-MN. After 8 days incubation at 37,5°C (+/- 0.5°C) substance is applied into the air cell. The hole is covered by a piece of adhesive tape. After 10,5 days of incubation blood was taken by incising in situ a major vessel. 6 µl of the obtained blood was spread out on slides immediately after blood sampling. After staining micronuclei were scored.

Substance	In vivo MNT	Comment / Ref.	HET-MN	Ref.
Ifosfamide	Positive	Heddle et al., 1983	Positive	Wolf and Luepke, 1997
Methotrexate	Positive	Heddle et al., 1983	Positive	Submitted to Mutation Research
Mitomycine C	Positive	CSGMT, 1992	Positive	Wolf and Luepke, 1997
Methylmethanesulfonate	Positive	CSGMT, 1992	Positive	Wolf and Luepke, 1997
N-Nitrosodiethylamine	Positive ^{liver}	A pro-mutagen Tates et al., 1980 ^{Liver}	Positive	Wolf et al., 2003
N-Nitrosodiethanolamine	Negative	Gilbert et al., 1981	Negative	Wolf et al., 2003
N-Nitrosodimethylamine	Positive	A pro-mutagen CSGMT, 1992	Positive	Wolf et al., 2003
Orange G	Negative	Kim and Margolin, 1999	Negative	Submitted to Mutation Research
Starch			Negative	Submitted to Mutation Research

Conclusion

- promising results: so far no false positives and no false negatives for ultimate mutagens as well as for pro-mutagens
- detection of liver-specific mutagens is possible
- further examination of more substances and different mode of action (e.g. aneugens) is needed
- next step: analyse transferability and inter-lab reproducibility

References

- 1 Wolf T, Luepke NP (1997). Formation of micronuclei in incubated hen's eggs as a measure of genotoxicity. *Mutat Res.*; 394(1-3):163-75.
- 2 Wolf T, Niehaus-Rolf C, Luepke NP (2002). Some new methodological aspects of the hen's egg test for micronucleus induction (HET-MN). *Mutat Res.*; 514(1-2):59-76.
- 3 Wolf T, Niehaus-Rolf C, Luepke NP (2003) Investigating genotoxic and hematotoxic effects of N-nitrosodimethylamine, N-nitrosodiethylamine and N-nitrosodiethanolamine in the hen's egg-micronucleus test (HET-MN). *Food Chem Toxicol.* 41(4):561-73.