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FROM: R. J. Kociba

REPORT ON INTERNATIONAL SYMPOSIUM ON CHROMOSOME ABBERATIONS BY INDUSTRIAL CHEMICAL AND VINYL CHLORIDE TOXICITY

OCTOBER 28-29, 1974, MILAN, ITALY

Principal Topics Covered in This Meeting Report:

1. Mutagenic Studies with Selected Compounds of Industrial Importance Other Than Vinyl Chloride

2. Mutagenic Studies with Vinyl Chloride

3. Carcinogenic Studies with Vinyl Chloride - by C. Maltoni

4. Description of "Vinyl Chloride Illness" - by P. L. Viola

DR. A. FORNI, UNIVERSITY OF MILAN CLINICA DEL LAVORA

PRESENTATION ON CHROMOSOME DAMAGE BY CHEMICAL AGENTS IN HUMANS
(See attached abstract)

Dr. Forni listed the following chemicals as capable of inducing chromosomal changes in humans: Ethyleneimine, DMSO, benzene, arsenic, lead, cyclamate, methylmercury and toluene-benzene. Principally a review of older data.

CHROMOSOME DAMAGE BY CHEMICAL AGENTS IN HUMANS

Alessandra FORNI

The assessment of chromosome aberrations in human metaphase cells is one of the methods for detecting a biological effect of chemical agents on the genetic material.

Numerous chemicals have been reported to induce chromosome changes in vivo and in vitro in mammalian cells. A short review of these groups of substances will be given, with special emphasis for the chemicals of industrial use. Personal data on chromosome changes observed in workers exposed to aromatic hydrocarbons and to lead will be presented, as examples of the usefulness of chromosomal studies in groups at risk. Some of the mechanisms possibly implicated in the production of chromosome alterations will be discussed.

Chromosome aberrations are indicative of a gross damage, which may result in cell death after some mitoses. The absence of visible chromosome changes, however, does not mean absence of genetic effects. Some recent more refined techniques, such as those producing «banding» patterns in chromosomes will probably enable us in future to detect more subtle alterations of the karyotype induced by exogenous agents.

DR. H. ZELLER, BASF

PRESENTATION OF THE MUTAGENIC EFFECTS OF CHEMICALS
IN THE EVALUATION OF THE TOXICOLOGICAL RISKS
(See attached abstract)

Dr. Zeller reviewed the tests for mutagenesis his BASF lab is using. This includes the (1) Host-mediated Assay in Mice, (2) Micronucleus Test in Mice, (3) Dominant Lethal Test in Rats, and (4) Cytogenetic Study of Bone Marrow Cells in Various Species.

ZUR PRÜFUNG DER MUTAGENEN WIRKUNG VON CHEMISCHEN STOFFEN IM RAHMEN DER TOXIKOLOGISCHEN RISIKOBEURTEILUNG
H. ZELLER

Die Aufgaben der modernen Toxikologie bei der Prüfung chemischer Stoffe werden umrissen. Als praktikable Basismethoden für den Nachweis mutagener Wirkungen im Rahmen der toxikologischen Risikobeurteilung werden der dominante Letaltest, in vivo cytogenetische Methoden, in vitro cytogenetische Methoden und der «hostmediated assay» an Hand von Beispielen besprochen. Es wir auf die Dosis/Wirkungsbeziehungen, auf das Zeitoptimum für den Nachweis der mutagenen Wirkung in den einzelnen Prüfsystemen, auf die unterschiedliche Empfindlichkeit der verschiedenen Prüfmethoden und auf die Reaktion generativer und somatischer Zellen gegenüber mutagenen Stoffen eingegangen.

Empfindlichkeit, praktische Anwendbarkeit und Relevanz der Ergebnisse für die toxikologische Risikobeurteilung werden diskutiert.

DR. KILLIAN - DOW

PRESENTATION ON COLLABORATIVE INTERLABORATORY STUDY
(See attached sheet)

Dr. Killian's presentation was well received by the group.

COLLABORATIVE STUDY TO MEASURE INTERLABORATORY VARIATIONS IN SCORING CHROMOSOME ABERRATIONS BY INDUSTRIAL CHEMICALS

D. Y. KILIAN

Six expert cytogenetic laboratories from the academic, industry, and government areas joined in a collaborative study to measure interlaboratory variation in cytogenetic analysis.

A preliminary workshop was held to resolve scoring differences and to develop a joint protocol and computer analytical techniques.

Using a common source of rats, Triethylene Melamine (TEM) at three dose levels was used and compared to control animals.

Cytogenetic analysis was accomplished with the scorer unaware of dosage.

Results were computerized on a standard 80 column IBM punch card and biostatistically evaluated.

Final results revealed an excellent correlation between interlaboratory scoring and also dose relationship.

DR. PEH, BASF

PRESENTATION ON STUDY OF MUTAGENIC EFFECTS OF ETHYLENEIMINE
(See attached abstract)

Dr. Peh described the BASF mutagenic studies of EI animals. Data were indicative EI was mutagenic under some test conditions.

PRÜFUNG VON ÄTHYLENIMIN AUF MUTAGENE WIRKUNG UNTER VERWENDUNG VERSCHIEDENER IN - VIVO - TESTMETHODEN
JUTTA PEH

Äthylenimin (Äl) wurde auf mutagene Wirkung im dominanten Letaltest, Mikronukleus-Test und bone-marrow-Test geprüft. Das Mutagen 2,3,5-Trisäthylenimino-benzochinon-(1,4) (Trenimon®, Handelsprodukt der Fa. Bayer, Leverkusen) wurde als positive Kontrollsubstanz in gleicher Weise untersucht. Als Versuchstiere dienten NMRI-Mäuse und Sprague-Dawley-Ratten.

Der dominante Letaltest wurde durchgeführt nach RÖHRBORN und VOGEL (1967) bzw. den Empfehlungen der WHO (1971), der Mikronukleus-Test nach BOLLER und SCHMID (1970) und der bone-marrow-Test nach «Report of the Ad Hoc Research» (1972). Die Dosis für Äl betrug im dominanten Letaltest bei einmaliger intraperitonealer Gabe 1,6 µl/kg Körpergewicht, im Mikronukleus-Test und bone-marrow-Test bei einmaliger oder 5maliger intraperitonealer Applikation 1,16 und 2,9 µl/kg Körpergewicht. Die Dosis für Trenimon® betrug im dominanten Letaltest 0,125 mg, im Mikronukleus-Test und bone-marrow-Test bei einmaliger intraperitonealer Gabe 0,05 mg bzw. 0,125 mg/kg und bei 5maliger intraperitonealer Gabe 0,025 bzw. 0,05 mg/kg Körpergewicht. Während Trenimon® in allen Testsystemen eine mutagene Wirkung zeigte, wurden nach Gabe von Äthylenimin keine sicheren Hinweise für eine mutagene Wirkung gefunden.

Versuchsdurchführung und Ergebnisse werden dargestellt und diskutiert.

DR. KILBEY, EDINBURGH UNIVERSITY

PRESENTATION ON ENVIRONMENTAL MUTAGENIC AGENTS
(See attached abstract)

Dr. Kilbey suggested the use of a "Tier" system of evaluating results of mutagenic tests. Initial tests would be conducted in the Ames bacterial system. Subsequent tests would be conducted with mammalian tissues. Risk/benefit evaluations would be used in dealing with positive results obtained with the testing of compounds vital for certain uses.

ENVIROMENTAL MUTAGENIC AGENTS - B.J. KILBEY

Genetic damage is fundamentally different from other types of damage. A brief summary will be given of the classes of genetic damage which probably constitute a hazard to the human population.

Although very few data exist from which the incidence of the various types of damage can be estimated for the population as a whole, it is nevertheless possible to discusse the probable consequences for the human population if there is an increase in the frequency of these different classes of demage.

Methods are available fo the assessment of the mutagenic potential of new compounds and some of these will be referrud to.

However, it is important that they be used in a rational way and that the results obtained can be translated into sensible policies regarding mutagenic agents.

These problems will be discussed.

DR. PEH, BASF

PRESENTATION ON MUTAGENIC STUDIES OF FORMAMIDE, ACETAMIDE, MONOMETHYLFORMAMIDE, DIMETHYLFORMAMIDE AND DIMETHYLACETAMIDE
(See attached abstract)

These compounds were negative when tested in the Dominant Lethal Test System.

PRÜFUNG VON FORMAMIDEN UND ACETAMIDEN AUF MUTAGENE WIRKUNG IM DOMINANTEN LETALTEST - JUTTA PEH

Die Säureamide Formamid (F), Acetamid (A), Monomethylformamid (MMF), Monomethylacetamid (MMA), Dimethylformamid (DMF) und Dimethylacetamid (DMA) wurden vergleichend auf mutagene Wirkung im dominanten Letaltest an männlichen NMRI-Mäusen geprüft. Das Mutagen 2,3,5-Tris-Äthylenimino-benzochinon-(1,4) (Trenimon®, Handelsprodukt der Fa. Bayer, Leverkusen) wurde als positive Kontrollsubstanz in gleicher Weise untersucht. Die Durchführung der vergleichenden Prüfungen erfolgte nach RÖHRBORN und VOGEL (1967) bzw. den Empfehlungen der WHO (1971). Die einmal intraperitoneal applizierten Substanzmengen entsprachen jeweils 1/5 LD5o und betrugen für F - 364 µl, A - 1700 mg, MMF - 560 µl, MMA - 700 µl, DMF - 400 µl und DMA - 680 µl bezogen auf kg Körpergewicht. Trenimon® wurde in einer Dosis von 0,125 mg/kg Körpergewicht verabreicht. Das injizierte Volumen betrug 100 µl/Tier. Die Auswertung der Untersuchungen erstreckte sich auf den klinischen Verlauf, Konzeptionsrate, durchschnittliche Zahl der Implantate und prozentualen Anteil abgestorbener Implantate (= Mutagenitätsindex).

Für jede der acht Paarungswochen wurden die Parameter Konzeptionsrate, durchschnittliche Zahl der Implantate und prozentualer Anteil abgestorbener Implantate mittels Chi^2-Test zwischen den einzelnen Versuchsgruppen hinsichtlich ihrer Signifikanz untersucht. Während Trenimon® eine starke mutagene Wirkung besaß, ließen die geprüften Säureamide keine Anzeichen einer mutagenen Wirkung in dem verwendeten Testsystem erkennen.

Versuchsdurchführung und Ergebnisse werden dargestellt und diskutiert.

DR. LEONARD, ITALY

PRESENTATION ON CYTOGENETIC HAZARDS OF LEAD AND CADMIUM
TO MAN AND CATTLE
(See attached abstract)

Dr. Leonard reported chromosomal changes in workers exposed to undetermined levels of Lead and Cadmium. No chromosomal changes were detected in cattle grazing on pasture adjacent to a metal refinery. Some cattle had died of heavy metal intoxication previous to this study.

CYTOGENETIC HAZARDS OF LEAD AND CADMIUM IN MAMMALS

A. LEONARD, GH. DEKNUDT

Clinical observations have been performed on peripheral blood lymphocytes of workers from zinc industry who presented signs of lead poisoning of different degrees as well as on peripheral blood lymphocytes off workers from cadmium industry.

Chromatid (gaps, breaks and exchanges) aberrations as well as chromosome aberrations (gaps, fragments, disturbances of spiralisation, rings and dicentrics) were observed in most of the workers. Negative results, however, were obtained on peripheral blood lymphocytes of cows intoxicated by the ingestion of hay which had been harvested near a plant refining metals. Six animals (on a total of 15) died within a few days and the lead content in the cadavers was markedly increased. Furthermore, chemical analysis performed on soils around the plant showed that the level of many other metals was very high (lead, 2-60 times higher than normal; chronium, ± 80 times; cadmium, ± 50 times; cobalt, ± 7 times; zinc, 50-400 times; copper, 12 times). The amount of arsenic in the grass was 50 to 200 times above normal. Nevertheless, no severe chromosome abnormalities were observed in the survivors.

The results are discussed in relation with the chromosome morphology of the two species.

DR. RANNUG, SWEDEN

PRESENTATION ON MUTAGENICITY OF VINYL CHLORIDE
(See attached publication and abstract)

Atmospheres containing 20% (V/V) Vinyl Chloride were mutagenic to Ames' Salmonella typhimurium TA1535. Metabolic activation (via addition of liver microsomes) was required in order to demonstrate mutagenic effects. The positive mutagenic effect seen in TA1535 is interpreted to indicate base substitution in DNA. Tests with strains TA1536, TA1537, and 1538 were negative.

Additional studies in the Host-mediated assay (mice exposed to 2% VC for 2.5 hrs.) and in the micromonucleus test (mice exposed to up to 10% VC for 1 hr.) have not shown mutagenic effects.

THE MUTAGENICITY OF VINYL CLORIDE AFTER METABOLIC ACTIVATION
U. RANNUG, A. JOHANSSON, C. RAMEL and C.A. MACHTMEISTER

In order to study the mutagenicity of vinyl chloride four different strains of Salmonella typhimurium has been used (TA 1535 - TA 1538). These auxotrophic strains revert to histidine independence either due to base-pair substitution (TA 1535) or base-pair addition or deletion (TA 1536 - TA 1538).

The bacteria were plated with or without liver cell preparations containing microsomes from rats according to Ames system.

Vinyl chloride caused point mutations, but only at the presence of liver microsomes, indicating that the mutagenic action of vinyl chloride requires a metabolic activation. From the data on the different strains of Salmonella it can be concluded that vinyl chloride causes base-pair substitution in DNA but no deletions or insertions of bases.

The results will be discussed in connection with data from preliminary experiments with different metabolites of vinyl chloride.