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Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing

A Bibliography with Abstracts

To Assist In:

- Refining Existing Test Methods
- Reducing Animal Usage
- Replacing Animals As Test Systems

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The Scientific Community, concerned about animal welfare, is sensitive to concerns regarding how and why animals are used in biomedical research and testing to evaluate the toxicological potential of various substances. Although alternatives to methods based on the use of animals may not satisfy all requirements and needs of the biomedical research and toxicologic testing communities, alternatives to the use of vertebrates are being developed and evaluated. Research on such methodologies is aimed at refining procedures to reduce pain and discomfort; reduce the number of animals required to provide scientifically valuable results; and to replace live vertebrates when an alternative methodology can be verified and validated by the scientific community.

The purpose of these bibliographies on "animal alternatives" is to provide a survey of the literature in a format which facilitates easy scanning. This bibliography includes citations from published articles, books, book chapters, and technical reports. Citations to items in non-English languages are indicated with [ ] around the title. The language is also indicated. Citations with abstracts or annotations relating to the method are organized under subject categories. This publication features citations which deal with methods, tests, assays or procedures which may prove useful in establishing alternatives to the use of intact vertebrates. Citations are selected and compiled through searching various computerized on-line bibliographic databases of the National Library of Medicine, National Institutes of Health.

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Suggestions and comments are welcome.

ALTERNATIVES TO ANIMALS
0
Hitchman N, Leaver M, George S. ALTERNATIVES TO WHOLE ANIMAL TESTING USE OF CDNA PROBES FOR STUDIES OF PHASE I AND II ENZYME INDUCTION IN ISOLATED PLAICE HEPATOCYTES. Seventh International Symposium on Responses of Marine Organisms to Pollutants (Primo 7), Goteborg, Sweden, April 20-22, 1993. Marine Environ Res 1994;39(1-4):289-92.

1
Reinhardt CA. THE SIAT RESEARCH TEACHING AND CONSULTING PROGRAM IN THE AREA OF IN VITRO TOXICOLOGY EXPERIMENTAL RESEARCH SCREENING AND VALIDATION. In: Reinhardt CA, editor. Alternatives to Animal Testing: New Ways in the Biomedical Sciences, Trends and Progress; Symposium; 1992 Nov 30; Zurich, Switzerland. New York: VCH Publishers, Inc: 1994. p. 89-98.

2
Spielmann H. HET-CAM TEST. Methods Mol Biol 1995;43:199-204.

3
Atterwill CK. ALTERNATIVE METHOD OF ASSESSING TOXICITY. Methods Mol Biol 1995;43:1-9.

4
Alarie Y, Nielsen GD, Andonian-Haftvan J, Abraham MH. PHYSICOCHEMICAL PROPERTIES OF NONREACTIVE VOLATILE ORGANIC CHEMICALS TO ESTIMATE RD50: ALTERNATIVES TO ANIMAL STUDIES. Toxicol Appl Pharmacol 1995;34(1):92-9.

This article presents the correlations obtained between the results on the potency of nonreactive airborne chemicals as sensory irritants and several of their physicochemical properties. The potency of airborne sensory irritants obtained from a reflexively induced decrease in respiratory frequency has been measured in the past using mice. Typically, their potency has been expressed as the exposure concentration necessary to decrease respiratory frequency by 50% (RD50). A large database of RD50 values is now available and such values are highly correlated with occupational exposure guidelines such as threshold limit values (TLVs). We used the nonreactive volatile organic chemicals from this database, for which relevant physicochemical variables are available or can be calculated. These variables were vapor pressure (P) or Ostwald gas-liquid partition coefficients (L). The liquids used for L values were n-hexadecane, octanol, N-formylmorpholine, tri-(2-ethylhexyl)phosphate, and olive oil. Excellent correlations were found between log RD50 and log P, as well as between log RD50 and log L16, log L(Oct), log L(NFM), log L(EHP), or log L(Oil). It follows that as an alternative to the bioassay, these physicochemical variables can be used to estimate RD50 of nonreactive volatile organic chemicals. Appropriate exceptions to general estimation of RD50 values from physicochemical variables are also presented, as well as the most appropriate estimates which can be obtained within homologous series.

5
Ehrich M. USING NEUROBLASTOMA CELL LINES TO ADDRESS DIFFERENTIAL SPECIFICITY

TO ORGANOPHOSPHATES. Clin Exp Pharmacol Physiol 1995;2(4): 291-2.

1. Organophosphates can cause acute toxicity, which follows inhibition of acetylcholinesterase (AChE), or delayed neuropathy, which follows inhibition of neuropathy target esterase (NTE). 2. Human neuroblastoma SH-SY5Y cells contain AChE and NTE. 3. Organophosphates actively able to inhibit AChE in animal models inhibited AChE in neuroblastoma cells. 4. Inhibition of NTE in neuroblastoma cells could identify active organophosphates capable of causing delayed neuropathy in animal models and distinguish these organophosphates from those that do not cause delayed neuropathy in animal models.

6
Sina JF, Galer DM, Sussman RG, Gautheron PD, Sargent EV, Leong B, Shah PV, Curren RD, Miller K. A COLLABORATIVE EVALUATION OF SEVEN ALTERNATIVES TO THE DRAIZE EYE IRRITATION TEST USING PHARMACEUTICAL INTERMEDIATES. Fundam Appl Toxicol 1995;26(1):20-31.

Much of the data which have been generated on in vitro alternatives to the Draize eye irritation test have dealt with compounds within a specific chemical class or product category. However, in the pharmaceutical industry, it is often necessary to evaluate materials which are not related in structure or properties. It was thus decided to evaluate a diverse series of chemicals in seven in vitro methods for estimating ocular irritation. Thirty-seven test materials were chosen to represent a broad range of pH, solubility, and in vivo irritation potential. Assays were chosen to include as many different types of end points as practical. The group of assays was composed of TOPKAT (assessing structure-activity relationships), bovine corneal opacity-permeability (BCO-P; corneal opacity/toxicity), Eytex (protein coagulation), neutral red uptake (cytotoxicity), MTT in living dermal equivalent (cytotoxicity), Microtox (cytotoxicity in bacteria), and CAMVA (inflammation/toxicity). The results of the study indicated that, in general, the cytotoxicity end points did not correlate well with the in vivo data. The BCO-P, CAMVA, and Eytex assays had the best overall concordance (88.9, 75.8, and 75.0%, respectively) with this set of compounds. Estimation of irritation potential based on structure-activity (TOPKAT) was possible for only approximately 50% of the compounds; however, the assay showed 100% sensitivity (i.e., no false negatives), but low specificity (i.e., negatives correctly identified only 54.5% of the time). These data suggest that for screening of chemicals of diverse structure and properties, the more mechanism-based assays, as opposed to general cytotoxicity assays, hold more promise and should be further evaluated.

7
Lang L. MOUSE OR MOLECULE? MECHANISM-BASED TOXICOLOGY IN CANCER RISK ASSESSMENT [NEWS]. Environ Health Perspect 1995;103(4):334-6.
Balls M. IN VITRO TESTS IN REGULATORY TOXICOLOGY: SYMPOSIUM CHAIRMAN'S SUMMING-UP. Arch Toxicol Suppl 1995;17:205-8.

8
Bass R. IN VITRO METHODS IN REGULATORY TOXICOLOGY. Arch Toxicol Suppl 1995;17:192-204.

9
Balls M. IN VITRO METHODS IN REGULATORY TOXICOLOGY: THE CRUCIAL SIGNIFICANCE OF VALIDATION. Arch Toxicol Suppl 1995;17:155-62.

10
Herzinger T, Korting HC,Maibach HI. ASSESSMENT OF CUTANIOUS AND OCULAR IRRITANCY: A DECADE OF RESEARCH ON ALTERNATIVES TO ANIMAL EXPERIMENTATION.
Fundam Appl Toxicol 1995;24(1):29-41.

Over the past decade increasing societal and scientific pressure has promoted the development of alternatives to local tolerance testing in laboratory animals. The use of isolated organs, fertilized hen's eggs, and cell culture systems has been proposed as well as the study of a toxicant's biochemical effects and structure-activity relationships. This paper critically reviews the current status of these approaches and discusses the preliminaries for the establishment of alternative methods in the process of safety assessment.

11
Prati M, Giavini E, Menegola E. ALTERNATIVES TO IN VIVO TESTS FOR TERATOLOGIC SCREENING. Ann Ist Super Sanita 1993;29(1):41-6.

During the last decade there has been a tremendous increase in publications describing methods for in vitro toxicological research and emphasizing their advantages, suitability and necessity, rather than the classical in vivo studies. In this review we shall look at and consider only a few short term tests, chosen on the basis of the particular relevance that they have in the evaluation of the substances: rodent whole embryo culture (for both pre- and post-implantation embryos), non-mammalian vertebrate embryo and invertebrate embryo cultures, organ culture, and at last cell culture. We have seen however that sometimes some of the developed methods suit neither the necessity of saving time, money, and animals, nor the consistency of the results in xenobiotic screening. The concomitant application of both in vivo and in vitro methodologies will improve the quality of teratological research, and therefore will contribute to a critical evaluation of developmental hazards.

12
Diener B, Abdel-Latif H, Arand M, Oesch F. XENOBIOTIC METABOLIZING ENZYME ACTIVITIES AND VIABILITY ARE WELL PRESERVED IN EDTA-ISOLATED RAT LIVER PARENCHYLAM CELLS AFTER CRYOPRESERVATION. Toxicol Appl Pharmacol 1995;130(1):149-53.

Rat liver parenchymal cells (PC) were isolated by EDTA perfusion and were purified by a subsequent Percoll centrifugation. The isolated PC had a viability of 95%, as judged by trypan blue exclusion. Freshly isolated PC were cryopreserved with an optimized protocol in a computer-controlled freezer. After thawing, the PC still retained a viability of 89%. The activities of representative xenobiotic metabolizing enzymes were compared between freshly isolated and cryopreserved PC after thawing. The cytochrome P450 content and the cytochrome P450 2C11 isoenzyme activity, determined by hydroxylation of

testosterone in intact cells, were not affected by the cryopreservation. The following phase II enzyme activities were also well maintained after cryopreservation: Phenol sulfotransferase (92%), 1-naphthol UDP-glucuronosyl transferase (95%), soluble epoxide hydrolase (87%), and glutathione S-transferase (88%), determined with broad spectrum substrate 1-chloro-2,4-dinitrobenzene. However, there was a significant decrease in plating efficiency between freshly isolated (86%) and cryopreserved (57%) PC when they were cultured. The initial quality of the freshly isolated PC is decisive for the success of cryopreservation. These results support the use of cryopreserved PC in pharmacology and toxicology with the aim to reduce the number of experimental animals used.

CARCINOGENESIS

13

Spitz MR, Hsu TC, Wu X, Fueger JJ, Amos CI, Roth JA. MUTAGEN SENSITIVITY AS A BIOLOGICAL MARKER OF LUNG CANCER RISK IN AFRICAN AMERICANS. Cancer Epidemiol Biomarkers Prev 1995;4(2):99-103.

Cigarette smoking is the major determinant of lung cancer. However, only a fraction of smokers develops lung cancer; genetically determined susceptibility factors seem to play an important role also. Previous case-control studies have shown that in vitro bleomycin-induced mutagen sensitivity is an independent risk factor for head-and-neck cancers, and preliminary data suggest a similar association with lung cancer. However, these studies were almost exclusively performed on Caucasian populations. To test whether ethnic differences in cancer risk are due to differences in mutagen sensitivity, we are using the in vitro mutagen sensitivity assay to conduct a case-control study of mutagen sensitivity and lung cancer risk in low-risk (Mexican-American) and high-risk (African-American) groups. Here we report the results of our ongoing study of 209 African-Americans (90 cases and 119 controls) in the Houston-Galveston area. Mexican-American data will be reported separately as case accrual increases. Predictably, all measures of cigarette smoking status (including intensity, duration, tar content, depth of inhalation, and type of cigarette) were significant predictors of risk. In addition, 55.3% of the cases were mutagen sensitive (defined as > or = 1 break/cell), compared with 24.6% of the controls, with an age-, sex-, and smoking-adjusted odds ratio (OR) of 3.7 (95% confidence limits = 1.4, 9.4). Of interest, higher risks were noted for former smokers (OR = 5.4) compared with current smokers (OR = 3.1) and especially for younger former smokers (< 55 years). By histologic-specific analysis, mutagen sensitivity was significantly associated with risk for adenocarcinoma (OR = 4.8) and squamous cell carcinoma (OR = 8.5).(ABSTRACT TRUNCATED AT 250 WORDS)

14

Jones TD. TOXICOLOGICAL POTENCY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN RELATIVE TO 100 OTHER COMPOUNDS: A RELATIVE POTENCY ANALYSIS OF IN VITRO AND
IN VIVO TEST DATA. Arch Environ Contam Toxicol 1995;29(1):77-85.

A common definition of relative potency is the dose of a reference compound

required to cause a particular incidence of a specific toxic response divided by the dose of a test compound needed to cause an equal incidence of that same effect. In this simple manner, toxicological assessments for a chemical of concern can be made in terms of another compound about which much is known from a human health perspective. Relative potency factors were used to compare 2,3,7,8-tetrachlorodibenzo-p-dioxin CAS # 1746-01-6 (TCDD) with 100 other compounds both individually and collectively. All results were standardized to a common scale that spanned many orders of magnitude and was indexed to an arbitrary potency of unity for benzo(a)pyrene (B(a)P). From comparisons between 2,771 pairs of bioassay results (i.e., matched experimental design conditions) for TCDD compared with the 100 other compounds, it was found that TCDD is about 600 times as toxic as B(a)P (interquartile range of 130 to 1,900). The distribution of relative potency values is fitted accurately with a log-normal distribution function having an untransformed ean of 550 and an untransformed slope (i.e., the inverse of the standard deviation of the distribution) of 140. These factors combined with (a) a reference lifetime carcinogenic risk level of 1/100,000 and (b) a universal, potency-dependent risk coefficient (estimated from the collection of epidemiologically-based carcinogens) yielded estimates that equally toxic concentrations for TCDD should be in the range of 13 pg/m3 and 7 pg/L in air and water, respectively.

15
Lichtenberg G, Nowak C, Gleier K, Meckert C, Richter-Reichhelm HB. ANCHORAGE INDEPENDENT COLONY GROWTH OF FETAL HAMSTER LUNG EPITHELIAL CELLS AFTER TREATMENT WITH DIEPOXYBUTANE. Toxicol Lett 1995;75(1-3): 193-9.

To test the reliability of a new cell transformation assay, a cloned fetal Syrian hamster lung epithelial cell line (M3E3/C3) was used. The target cells originating from the respiratory tract were treated in vitro over a concentration range of 0-10-5 M/l with diepoxybutane, cultured during the expression period of 28 or 35 days and then transferred into soft agar. Anchorage independent colony growth in soft agar occurs only if cells are transformed. Growth and number of colonies were taken as a score of the carcinogenic potential of the test substance. Under the conditions of this cell transformation assay it was possible to detect the carcinogenic potential of diepoxybutane unequivocally.

16
Miyasaka K, Ohtake K, Nomura K, Kanda H, Kominami R, Miyashita N, Kitagawa T. FREQUENT LOSS OF HETEROZYGOSITY ON CHROMOSOME 4 IN DIETHYLNITROSAMINE-INDUCED
C3H/MSM MOUSE HEPATOCELLULAR CARCINOMAS IN CULTURE. Mol Carcinog 1995;13(1):37-43.

Genetic changes, in particular the loss of heterozygosity (LOH) and the presence of c-Ha-ras codon 61 point mutations, were investigated in diethylnitrosamine-induced hepatocellular carcinomas (HCCs) in C3H/MSM F1 mice. (MSM are wild mice.) LOH analysis of 48 primary tumors with microsatellite probes covering at least one proximal and one distal site of each autosome revealed no obvious positive results for LOH. Analysis of 23 cell lines established from seven of these HCCs, however, showed LOH on

chromosome 4 in all (seven of seven), even in early passages (G2-G3). With regard to other chromosomes, LOH was observed only rarely on chromosomes 16 and 19. These allelotype features were maintained in later passages (G11-G14), with only a few additional occurrences of LOH appearing on chromosomes 1, 6, and 8. Extensive analyses with multiple microsatellite probes from chromosome 4 and with 52 cell lines established from 24 HCCs of 18 mice revealed LOH in 22 of the tumors (92%), with the shortest region about 10 cM distal to the alpha-interferon gene. No c-Ha-ras oncogene activation in codon 61 was observed. These data indicate that loss of tumor suppressor genes on chromosome 4 may play an important role in mouse hepatocarcinogenesis in progression in vivo or in immortalization in vitro or both.

17
Cunningham ML, Elwell MR, Matthews HM. CORRECTION OF PREVIEWS 97552935. RELATIONSHIP OF CARCINOGENICITY AND CELLULAR PROLIFERATION INDUCED BY MUTAGENIC NONCARCINOGENS VS CARCINOGENS: III. ORGANOPHOSPHATE PESTICIDES VS
TRIS(2,3-DIBROMOPROPYL)PHOSPHATE. CORRECTION OF TITLE FROM RELATIONSHIP OF
CARCINOGENICITY AND CELLULAR PROLIFERATION INDUCED BY MUTAGENIC NONCARCINOGENS
VS CARCINOGENS: III. ORGANOPHOSPHATE PESTICIDES VS TRIS(2,3-DIBROMOPROFYL)-PHOSPHATE. Fundam Appl Toxicol 1994;23(3):363-9.

Our laboratory has been examining the mechanisms whereby chemicals produce mutagenicity in short-term in vitro assays yet fail to produce carcinogenesis in 2-year rodent bioassays. Previous studies indicated that some mutagenic hepatocarcinogens increased cell proliferation in the target organ, the liver, while other structurally related mutagens that were noncarcinogenic failed to do so. We demonstrate in this report that another mutagenic carcinogen, tris(2,3-dibromopropyl)phosphate, increased cell proliferation that was localized in the outer medulla of the kidney. This was also the target site for carcinogenesis in a 2-year bioassay and is another example of the association between chemically induced cell proliferation and carcinogenesis. This study also reports the absence of increased cell proliferation in the liver or kidney after exposure in the diet to the mutagenic organophosphate insecticides dimethoate, dioxathion, and dichlorvos following dietary exposure for 2 weeks at the same dose levels and routes of exposure that did not increase the tumor incidence in either organ in 2-year carcinogenesis assays. The present studies support the tenet that chemically induced cell proliferation may be a necessary prerequisite for chemical carcinogenesis, since in rat liver and kidney there was neither cell proliferation after 2 weeks nor tumor development after 2 years dietary exposure to the mutagenic organophosphate insecticides dimethoate, dioxathion, and dichlorvos.

18
Silverman JA, Hill BA. CHARACTERIZATION OF THE BASAL AND CARCINOGEN REGULATORY ELELMENTS OF THE RAT MDR1B PROMOTER. Mol Carcinog 1995;13(1):50-9.

In this report we characterized the transcriptional regulation of the rat mdr1b gene by xenobiotics. The expression of this gene was increased in

primary rat hepatocytes and in the H4-II-E hepatoma cell line by exposure to carcinogens such as aflatoxin B1, N-acetoxy-2-acetylaminofluorene, and methyl methanesulfonate. Nuclear run-on experiments indicated that the higher steady-state levels of mdr1b mRNA were due to an increase in transcription. The 5'-flanking region of the mdr1b gene was isolated, sequenced, and functionally characterized in transient and stable transfection assays. A single transcription start site was identified for this gene; no alternate start sites were used after induction with aflatoxin B1. Deletion analysis of this promoter demonstrated that the sequence between nt -214 and -178 was critical for basal promoter activity. This region did not contain any consensus-binding sites for previously identified transcription factors. A negative regulatory region was also identified between nt -940 and -250. No specific carcinogen-responsive element was identified; the xenobiotic response required a large part of the promoter. These data suggest that the carcinogen induction of mdr1b expression is mediated through sequences that overlap or that are identical to the basal promoter element.

19
Pintao AM, Pais MS, Coley H, Kelland LR, Judson IR. IN VITRO AND IN VIVO ANTITUMOR ACTIVITY OF BENZYL ISOTHIOCYANATE: A NATURAL PRODUCT FROM TROPAEOLUM
MAJUS. Planta Med 1995;61(3):233-6.

Cultured cells of Tropaeolum majus produce significant amounts of benzyl glucosinolate which, through enzymatic hydrolysis, results in the production of benzyl isothiocyanate (BITC). This study reports on the in vitro anticancer properties of BITC against a variety of human and murine tumor cell lines by four independent methods; SRB, MTT, cell counting, and clonogenic assays. Regardless of the assay used, BITC showed promising cytotoxicity in the low micromolar range (0.86 to 9.4 microM) against four human ovarian carcinoma cell lines (SKOV-3, 41-M, CHI, CHIcisR), a human lung tumor (H-69), a murine leukemia (L-1210), and a murine plasmacytoma (PC6/sens). The L1210 cells were most sensitive. BITC administered to mice bearing the ADJ/PC6 plasmacytoma subcutaneous tumor showed toxic effects at a dose of 200 mg/kg (within 24 h of drug administration) but no reduction in tumor mass. However, the growth inhibitory properties of BITC against a range of tumor cell types warrant further in vivo anti-tumor evaluation as well as its biotechnological production.

20
Segerback D, Calleman CJ, Schroeder JL, Costa LG, Faustman EM. FORMATION OF N-7-(2-CARBAMOYL-2-HYDROXYETHYL)GUANINE IN DNA OF THE MOUSE AND THE RAT FOLLOWING INTRAPERITONEAL ADMINISTRATION OF [14C]ACRYLAMIDE. Carcinogenesis 1995;16(5):1161-5.

Acrylamide is an alkylating agent which reacts very slowly in direct reactions with DNA and is negative in the Ames test, but is carcinogenic in mice and rats. In order to explain the cancer-initiating properties of acrylamide we have studied DNA adduct formation in vitro with a metabolizing system and in vivo in mice and rats following i.p. administration of 14C-labeled acrylamide. A major adduct found in both species was

N-7-(2-carbamoyl-2-hydroxy-ethyl)guanine, formed by reaction of the DNA with the epoxide metabolite glycidamide. The levels of this adduct were similar in the different organs of the two rodent species, which supports the notion that glycidamide is relatively evenly distributed among tissues and that the organ-specificity in acrylamide carcinogenesis cannot be explained by a selective accumulation of the DNA-reactive metabolite in target organs.

21
Hiraga S, Arita N, Ohnishi T, Izumoto S, Taki T, Yamamoto H, Higuchi M, Hayakawa T. TRANSFORMATION OF TYPE 1 ASTROCYTES WITH N-ETHYL-N-NITROSOUREA:
ESTABLISHMENT OF AN IN VITRO SYSTEM AND THE ROLE OF THE p53 GENE. Glia 1995;13(1):51-63.

N-ethyl-N-nitrosourea (ENU)-induced gliomas, animal models of human gliomas, are most frequently oligodendrocytic, while human gliomas tend to be astrocytic. To facilitate a detailed study of human glial carcinogenesis, we developed an in vitro system using type 1 astrocyte transformation with ENU. Type 1 astrocytes from fetal Wistar rat brain were treated by a single dose of ENU. Transformed colonies appeared 50 days after exposure to single doses of ENU greater than 150 micrograms/mL. Cloned cells from these colonies retained the immunohistochemical characteristics of type 1 astrocytes. They showed rapid growth and high saturation densities, colony formation in low (2%) serum medium and gave rise to tumors when injected into nude mice. When p53 expression was studied at each passage, a single cell positive for mutant p53 protein emerged 40 days after ENU treatment. In the next 1-3 passages, the mutant p53 positive cell formed piled-up colonies and exhibited dominant growth. Northern blot analysis showed markedly increased accumulations of p53 mRNA in transformed cells. This in vitro transformation system of type 1 astrocytes provides a valuable tool for further investigations of astrocyte carcinogenesis.

22
Swanson SM, Guzman RC, Collins G, Tafoya P, Thordarson G, Talamantes F, Nandi S. REFRACTORINESS TO MAMMARY CARCINOGENESIS IN THE PAROUS MOUSE IS REVERSIBLE BY HORMONAL STIMULAITON INDUCED BY PITUITARY ISOGRAFTS. Cancer Lett
1995;90(2):171-81.

We have previously reported that mouse mammary epithelial cells transformed in vitro yield tumors which vary qualitatively and quantitatively as a function of the mitogenic environment in which the cells are propagated at the time of carcinogen treatment. One milieu supportive of transformation in vitro was medium supplemented with progesterone and prolactin as the mitogens. We have performed parallel studies in which virgin mice were isografted with pituitaries resulting in elevated serum titers of progesterone and prolactin. After carcinogen treatment, these mice developed mammary tumors which included those identical genotypically and phenotypically to tumors induced in vitro in cells grown in progesterone and prolactin during carcinogen exposure. Our current working hypothesis is that the mitogenic environment around the time

of carcinogen administration can modulate the incidence and phenotype of the resultant tumors. To further test this hypothesis, we have evaluated the susceptibility of hormonally-stimulated parous mice to chemically induced mammary carcinogenesis since parity is known to significantly reduce the susceptibility of the mouse mammary gland to carcinogenesis. Virgin or multiparous BALB/c mice were isografted with two pituitaries. Five weeks after surgery, the mice were injected with N-methyl-N-nitrosourea (MNU; 50 micrograms/g i.v.). Mammary carcinomas arose in 85% (11/13) with a median latency of 22.8 weeks and 1.9 tumors per virgin mouse and 80% (24/30) with a median latency of 22.1 weeks at a frequency of 1.9 tumors per parous mouse. Only 14% (2/14) of the non-isografted, age-matched parous controls developed tumors when injected with MNU. Fourteen parous mice receiving only pituitary isografts (no MNU), did not develop mammary carcinomas within the 7-month period of the study. These results demonstrate that parous BALB/c mice are refractory to MNU-induced mammary carcinogenesis and that this refractoriness is not permanent, but can be overcome by hormonal stimulation mediated by pituitary isografts.

23
Park NH, Gujuluva CN, Baek JH, Cherrick HM, Shin KH, Min BM. COMBINED ORAL CARCINOGENICITY OF HPV-16 AND BENZO(A)PYRENE: AN IN VITRO MULTISTEP CARCINOGENESIS MODEL. Oncogene 1995;10(11):2145-53.

We previously immortalized normal human oral keratinocytes by transfection with recombinant HPV-16 DNA and subsequently exposed the cells to benzo(a)pyrene for 7 days. The exposure to benzo(a)pyrene modified the immortalized cells: the modified cells (HOK-16B-BaP) proliferated in an ordinary culture medium containing physiological calcium level (1.5 mM), but demonstrated only enhanced proliferation capacity without tumor formation in nude mice and failed to show in vitro anchorage-independency. In this study, we further modified the HOK-16B-BaP cells by subculturing the cells in a medium containing benzo(a)pyrene for 6 months. The cells were further modified with a chronic benzo(a)pyrene exposure and were termed HOK-16B-BaP-T cells (1) demonstrated a malignant phenotype in organotypic 'raft' culture, (2) showed in vitro anchorage-independency, (3) developed tumors in nude mice when injected subcutaneously, (4) contained a significantly higher copy number of intact and integrated HPV-16 DNA; (5) contained higher level of HPV-16 E6/E7 messages and E7 protein, (6) were more resistant to transforming growth factor-beta1 and (7) secreted higher level of vascular endothelial growth factor with molecular weight of 56 kd than parental HOK-16B-BaP cells. However, the levels of p53 and ras proteins and the levels of p53, c-myc and c-fos transcripts in the HOK-16B-BaP-T cells were not different from those in the HOK-16B-BaP cells. The highly conserved coding regions of the p53, c-Ha-ras1, and c-Ki-ras2 genes of the tumor cells were not mutated. These data indicate that the HPV-immortalized human oral keratinocytes can convert to tumorigenic cells by chronic exposure to benzo(a)pyrene. The tumorigenic conversion seems to be associated with (1) the overexpression of viral oncogenes such as E6 and E7 genes, (2) the higher resistance of cells to transforming growth factor-beta1 and (3) the high secretion of 56 kd vascular endothelial growth factor from the cells.

CELL, TISSUE AND ORGAN TOXICITY

24

Murphy JE, Janszen DB, Gargas ML. AN IN VITRO METHOD FOR DETERMINATION OF TISSUE PARTITION COEFFICIENTS OF NON-VOLATILE CHEMICALS SUCH AS 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND ESTRADIOL. J Appl Toxicol 1995;15(2):147-52.

The development of an in vitro vial equilibration technique for determining tissue and liquid partition coefficients for non-volatile chemicals is described. Radiolabeled chemical dissolved in propylene carbonate is equilibrated with tissues or liquid at 37 degrees C in a vial system. The solvent must be essentially immiscible with the test material. The amount of chemical movement to the tissue or liquid is compared to an appropriate reference vial, and tissue or liquid:solvent partition coefficients are calculated. Tissue:solvent values divided by blood:solvent values provide tissue:blood partition coefficients required for developing physiologically based pharmacokinetic models for chemicals. These models are useful for estimating internal tissue doses to assess human risk from exposure to chemicals. Partition coefficients for various rat tissues, 0.9% saline solution and olive oil were determined in this study for radiolabeled 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and for the less fat-soluble compound, estradiol. The TCDD tissue:propylene carbonate partition coefficients were found to be: blood, 0.091; fat, 17.02; liver, 0.419; brain, 0.632; kidney, 0.305; muscle, 0.408. For estradiol, the tissue:propylene carbonate partition coefficients were: blood, 0.286; fat, 0.169; liver, 1.032; brain, 0.554. The TCDD results compared well with values reported and estimated from a more protracted in vivo approach. Thus, this current technique offers a simpler and time-saving alternative to in vivo approaches for determining the partition coefficients of non-volatile compounds.

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Clothier RH. THE FRAME CYTOTOXICITY TEST KENACID BLUE. Methods Mol Biol 1995;43:109-18.

26

Cheung RC, Gray C, Boyde A, Jones SJ. EFFECTS OF ETHANOL ON BONE CELLS IN VITRO RESULTING IN INCREASED RESORPTION. Bone 1995;16(1):143-7.

Abuse of alcohol has been found to be an important risk factor for fractures and osteoporosis, and tissue culture experiments have indicated that low concentrations of ethanol can affect bone formation and resorption. This study investigated direct effects of ethanol on bone cells using an in vitro resorption assay. Osteoclasts from long bones of 19-day prehatch chicks were seeded onto slices of dentine and cultured with control medium alone, or medium containing 0.001%, 0.01% or 0.1% ethanol, at 37 degrees C with 5% CO2 for 24 h before being removed. The volumes and areas of resorption pits made in the dentine were measured using confocal laser reflection microscopy (Lasertech 1LM21 system) and the pits counted. An increase in the pit numbers and mean pit areas, volumes and volume/area ratios was observed with 0.001% and 0.01% ethanol, with a dose-related, bell-shaped curve of resorption. Greatest mean volume resorbed per pit ($p < 0.05$), mean area resorbed per pit

($p < 0.01$) and number of pits was at 0.01% ethanol. Volume/area (mean depth) per pit was greatest at 0.001% ethanol ($p < 0.05$). This study has shown that ethanol, even at blood concentrations experienced by the social drinker, has an immediate direct effect on bone cells in vitro, resulting in increased resorption by osteoclasts.

27
Zwahlen RD, Holden WJ, Wyder-Walther M, Holub M, Moiola F. INFLUENCE OF ANTI-INFLAMMATORY DRUGS ON ADHESION OF NEUTROPHILS TO ENDOTHELIAL CELLS CUTLURED ON MICROCARRIERS: A NOVEL IN VITRO SYSTEM AS AN ALTERNATIVE TO ANIMAL
EXPERIMENTATION. Zentralbl Veterinarmed A 1994;41(9):671-82.

Pharmacological control of inflammation by steroidal (SAIDs) and nonsteroidal (NSAIDs) antiinflammatory drugs is of substantial clinical importance. To reduce the number of animals used in pharmacological and toxicological evaluation of these drugs we developed a novel assay to determine adhesion of bovine neutrophils (PMN) to bovine aortic endothelial cells (BAEC) cultured on microcarriers in a flow-through system. Pretreatment of BAEC with thrombin (10(-7)-10(-4) M) led to a dose-dependent increase of PMN-adhesion (10(-6)-10(-4) M:$P < 0.05$); platelet-activating factor (10(-9) M) and 1:200 diluted zymosan-activated serum (ZAS) had similar effects ($P < 0.001$). Pretreatment of PMN with SAIDs (50.9 and 509 microM dexamethasone, 12.2 and 24.4 microM flumethasone) did inhibit adhesion to ZAS-treated BAEC dose-dependently. Pretreatment of PMN with NSAIDs had a less consistent influence on adhesion to ZAS-stimulated BAEC. While phenylbutazone (0.33 and 3.3 mM), diclofenac (0.392 and 0.574 mM), indomethacine (0.436 and 0.872 mM), and acetylsalicylic acid (3.47 and 16.94 mM) induced dose-dependent inhibition of PMN-adhesion to ZAS-treated BAEC, piroxicam (0.377 and 0.754 mM) inhibited PMN-adhesion strongly ($P < 0.001$) but not dose-dependently, and ketoprofene (0.614 and 1.228 mM) had no effect on PMN-adhesion. The method presented here is efficient for evaluating the pharmacological modulation of PMN interaction with endothelial cells, and useful for studying further aspects of endothelial cell biology.

28
Kuehn U, Empertz U, Knop J, Becker D. A NEW METHOD FOR PHENOTYPING PROLIFERATING CELL NUCLEAR ANTIGEN POSITIVE CELLS USING FLOW CYTOMETRY: IMPLICATIONS FOR ANALYSIS OF THE IMMUNE RESPONSE IN VIVO. J Immunol Methods 1995;179(2):215-22.

The incorporation of radioactive nucleotides into newly synthesized DNA has been established as a standard method for the detection of proliferation in eucaryotic cells. Unfortunately the use of this method makes it harder to obtain information on the phenotype of proliferating cells in mixed cell populations. For this reason we established a flow-cytometric approach employing a monoclonal antibody specific for murine as well as human proliferating cell nuclear antigen (PCNA) and a double labeling technique for detection of cell membrane-expressed phenotypic markers. The efficiency of this immunostaining procedure was confirmed by simultaneous and highly specific detection of PCNA in nuclear structures as well as cell

membrane-expressed antigens using cytological techniques. In vitro experiments with mitogen- and alloantigen-stimulated murine lymph node cells (LNC) and human peripheral blood mononuclear leukocytes (PBML) revealed a good correlation of total (3H)thymidine incorporation into DNA and expression of PCNA. For the analysis of proliferating cells activated in vivo the method was employed to evaluate the local lymph node assay which assesses the allergenicity of small chemicals. LNC prepared from the cervical lymph nodes of mice treated on 4 consecutive days with sensitizing concentrations of the contact allergens oxazolone, TNCB and DNFB as well as the irritants benzoic acid and SLS in comparison to the solvent control showed a dramatic increase in the total amount of proliferating cells for contact allergen-treated animals in comparison to the solvent control and irritant-treated mice. In addition a detailed phenotyping of the proliferating cell populations was possible. This approach offers an easy to perform, non-radioactive method for the assessment of proliferation of murine as well as human leukocytes in vitro and especially in vivo and will be of great advantage for situations where the phenotype of proliferating cellular subsets in heterogeneous populations is of interest.

29
Marinovich M, Guizzetti M, Ghilardi F, Paoletti R, Galli CL. EFFECT OF 25-HYDROXYCHOLESTEROL, 26-HYDROXYCHOLESTEROL AND ITS ANALOGUE, 26-AMINOCHOLESTEROL, ON PROTEIN CONTENT, PROTEIN SYNTHESIS AND LDH LEAKAGE IN
MOUSE EPIDERMAL CELLS. Toxicology 1995;99(1-2):125-34.

The cytotoxicity of 25-hydroxycholesterol, 26-hydroxycholesterol and its analogue, 26-aminocholesterol was investigated in murine epidermal cell line, HEL-30. Lactate dehydrogenase (LDH) leakage, protein synthesis and protein content were determined after exposure of cell monolayers to the compounds ranging from 0.1-200 muM for 2, 6 or 24 h. 26-Aminocholesterol affected all the parameters studied time and concentration dependently; 25hydroxycholesterol and 26-hydroxycholesterol were not toxic for HEL-30 cells. The cellular target for 26-aminocholesterol was primarily the membrane, since the LDH leakage was already detectable 10 min after exposure. On the other hand, for the other two oxysterols a protective role on this structure might be postulated. In fact 25- hydroxycholesterol and 26-hydroxycholesterol decreased the natural LDH leakage due to the ageing of the culture. In addition, 20 AM 25-hydroxycholesterol reversed the effect of moderately lytic doses of 26-aminocholesterol and Triton X-100, but not of sodium dodecyl sulfate.

30
Vogel C, Abel J. EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON GROWTH FACTOR EXPRESSION IN THE HUMAN BREAST CANCER CELL LINE MCF-7. Arch Toxicol 1995;69(4):259-65.

The aim of this study was to examine whether changes in growth factor or cytokine expression could be responsible for the growth inhibitory effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the human breast cancer MCF-7 cell line. Treatment of MCF-7 cells with 10 nM TCDD for 7 days reduced the

cell growth to 60% of control; this effect was partly abolished by cotreatment of the cells with 100 nM 17beta-estradiol (E2). The inhibition of cell growth by TCDD was accompanied by an enhanced secretion of transforming growth factor-beta (TGF-beta) and the TGF-beta content in cell culture supernatants was 2-fold higher than in controls. Using reverse transcription polymerase chain reaction (RT-PCR), the effect of TCDD on the expression of TGF-beta isoforms, transforming growth factor-alpha (TGF-alpha), tumor necrosis factor-alpha (TNF-alpha) and interleukin-1beta (IL-1beta) was investigated. It was demonstrated that incubation with 1, 10 and 100 nM TCDD for 24 h increased mRNA levels IL-1beta. The strongest effect was found on IL-1beta, the mRNA level of which was dose-dependently increased. TCDD had a minor effect on TGF-alpha and TNF-alpha mRNA. The mRNA levels were significantly increased after treatment with 10 and 100 nM TCDD. The mRNA expression of TGF-beta1 and TGF-beta2 was unchanged, whereas the TGF-beta3 mRNA level was enhanced 2 to 3-fold after TCDD treatment. From the results, we suggest that TCDD-induced growth inhibition in MCF-7 cells is related to the growth action of a set of growth factors and cytokines which have a contextual action on MCF-7 cell proliferation.

31
Lee J , Green MH, Amiel D. SYNERGISTIC EFFECT OF GROWTH FACTORS ON CELL OUTGROWTH FROM EXPLANTS OF RABBIT ANTERIOR CRUCIATE AND MEDIAL COLLATERAL
LIGAMENTS. J Orthop Res 1995;13(3):435-41.

Cellular migration and proliferation are integral aspects of wound healing. An in vitro assay for cellular migration and proliferation using explants of rabbit anterior cruciate and medial collateral ligaments was developed previously. This study presents the effects of serum-free culture medium supplemented with basic fibroblast growth factor, bovine insulin, transforming growth factor-beta 1, and platelet-derived growth factor-B, added either individually or in combination, on cell outgrowth in explants of rabbit anterior cruciate and medial collateral ligaments. Outgrowth was assessed at 3 and 6 days by counting the number of cells surrounding the tissue explants. For explants of both ligaments, cell outgrowth was dependent on the presence of 10% fetal bovine serum or the combination of growth factors. Little outgrowth occurred in explants of either ligament in the presence of basic fibroblast growth factor, transforming growth factor-beta 1, or bovine insulin. Platelet-derived growth factor-B at concentrations of 20 and 100 ng/ml seemed to increase cell outgrowth from medial collateral ligament explants at 6 days. The outgrowth from the explants of both ligaments was much greater in the presence of all four growth factors than the sum of the outgrowth with the individual factors. This synergistic effect was as much as 10-fold and 20-fold for the anterior cruciate ligament explants at days 3 and 6, respectively, but no more than 3-fold for the medial collateral ligament explants at these times. Medial collateral ligament explants exhibited greater cell outgrowth than anterior cruciate ligament explants in 10% serum and in the presence of the four growth factors.

32
Chakradeo PP, Nair J, Bhide SV. METABOLISM OF N'-NITROSONORNICOTINE BY ADULT

AND FETAL HUMAN OESOPHAGEAL CULTURES. Cell Biol Int 1995; 19(1):53-8.

The metabolism of (3H) labelled N-Nitrosonomicotine a major constituent of the class of Tobacco Specific Nitrosamines was studied in adult and fetal human oesophageal cultures. The metabolites were separated by HPLC and were identified when compared to the standards as OH-acid from 5'-hydroxylation, NNN-1-N-oxide formed via the pyridine N-oxidation pathway and Keto acid from 2'-hydroxylation in both the adult and fetal cultures. These results indicate that hydroxylation which leads to electrophilic diazohyroxides is the major pathway of NNN metabolism in cultured human oesophagus. In the adult cultures levels of metabolites formed were 20.32 pmoles/mug DNA of OH acid 11.08 pmoles/mug DNA of NNN-1-N-oxide and 7.6 7 pmoles/mug DNA of Keto acid. In the fetal cultures levels were 10.85 pmoles/mug DNA of OH acid, 9.40 pmoles/mug DNA of NNN-1-N-oxide and 7.91 pmoles/mug DNA of Keto acid. These results indicate that alpha-hydroxylation is the key step in the metabolic activation of NNN in human oesophagus.

33
Datta K, Joseph P, Roy SK, Srinivasan SN, Kulkarni AP. PEROXIDATIVE XENOBIOTIC OXIDATION BY PARTIALLY PURIFIED PEROXIDASE AND LIPOXYGENASE FROM HUMAN FETAL
TISSUES AT 10 WEEKS OF GESTATION. Gen Pharmacol 1995;26(1):107-12.

1. Present study reports the ability of partially purified peroxidase and lipoxygenase from human fetal tissues at 10 weeks of gestation to oxidize selected xenobiotics in vitro. 2. Peroxidase was found to oxidize four different chemicals in the presence of H2O2. Sodium azide and potassium cyanide inhibited peroxidase activity towards guaiacol in a concentration-dependent manner. 3. The dioxygenase and co-oxidase activities of lipoxygenase towards linoleic acid and four model xenobiotics, respectively, were observed. Both the catalytic activities of lipoxygenase were significantly inhibited by < 1.0 muM nordihydroguaiaretic acid. 4. These findings suggest that peroxidase and lipoxygenase may be important pathways for peroxidative xenobiotic oxidation in human fetal tissues.

34
Grote JJ, Hesseling SC, Tjebbes GJ, Van Blitterswijk CA. EFFECT OF HA-1A MONOCLONAL IGM ANTIBODY ON ENDOTOXIN-INDUCED PROLIFERATION OF CULTURED RAT
MIDDLE EAR EPITHELIUM. Ann Otol Rhinol Laryngol 1995; 104(3):226-30.

The effect of human monoclonal antibody HA-1A (Centoxin) on the effect of endotoxin on cultured rat middle ear epithelium was investigated. The addition of endotoxin to the standard culture medium revealed a concentration-related proliferative effect on cultured mt middle car epithelium, leading to cobblestone cells, cell tracks, and stratification of epithelium, whereas rat middle ear epithelium cultured in standard medium grew as a monolayer composed of flat polygonal cells. Addition of HA-1A to standard medium supplemented with endotoxin gave rise to a statistically significant suppression of the proliferative effects of endotoxin on these cells. The morphology of rat middle ear epithelium cultured in the presence of HA-1A and endotoxin showed

that these cells still had a tendency to form cobblestone-like cells and cell tracks, but to a substantially lower degree. The present results support the hypothesis that HA-1A suppresses the proliferative and morphological effects of endotoxin on rat middle ear epithelium and may play an important role in the pathogenesis of otitis media.

35
Muraki Y, Yamada M, Nii S, Kumon H, Ohmori H. [IN VITRO ASSESSMENT OF RELATIVE PHOTOTOXICITY OF QUINOLONE ANTIBACTERIAL AGENTS BY REDUCTION OF NEUTRAL RED UPTAKE IN CULTURED CELLS.] Nippon Kagaku Ryoho Gakkai Zasshi 1995;43(3):357-60. (Jpn)

The relative phototoxicity of 10 antibacterial drugs in the quinolone group was detd. by an in vitro assay, in which redn. of neutral red uptake was used as a marker of cell injury. Human embryonal lung fibroblasts or Vero cells derived from a green monkey kidney were incubated with potential phototoxins. The cell cultures were irradiated with long wave-length UV, and the capacity for neutral red uptake was detd. The phototoxicity of 10 quinolones was much lower than that of doxycycline, a known photosensitizer. Among them, enoxacin, lomefloxacin, ciprofloxacin, ofloxacin, and nalidixic acid demonstrated higher phototoxicity, suggesting a good correlation with the clin. occurrence of photosensitivity. Norfloxacin, balofloxacin (Q-35), AM-1155, and T-3761 had less potent phototoxicity. This methodol. may provide a useful rapid method to quantitate the phototoxic potential of newly developed drugs.

36
Dumitrescu M, Jucu V, Zaharia CN, Belu O, Rojanschi D, Diaconu C, Talos D, Panaitescu M. [ACTION OF SOME AMPHIPHILIC DRUGS ON HUMAN FIBROBLASTS IN VITRO.
POSSIBLE ANTIVIRAL AND ANTITUMOR USES.] Rev Roum Virol 1993;44(3-4):211-21. (Fre)

The release of lactic dehydrogenase (LDH) from human embryo fibroblasts in vitro was used as a test of membrane stability. Two amphiphilic drugs, metomidate and timolol, stabilized the membrane, as shown by a reduced release of LDH. Another drug, Ca dobesylate, had the opposite effect, making the membrane less stable. The use of the LDH test for the selection of some natural complexes or synthetic drugs with membrane-stabilizing and hence potential antiviral activity (by inhibiting virus penetration into cells) is proposed. Furthermore, LDH activity was inhibited by metomidate, causing the intracellular accumulation of lactate and consequent lowering of intracellular pH. The use of metomidate for potentiating the action of classical antitumor drugs, lowering tumor cell pH, is proposed.

37
O'Hare S, Atterwill CK, editors. METHODS IN MOLECULAR BIOLOGY. Vol 43, IN VITRO TOXICITY TESTING PROTOCOLS. Totowa (NJ): Humana Press Inc; 1995. 332 p.

38
Langner A, Melzig MF, Kempa S, Krause A. [THE USE OF LYMPHOCYTE CULTURES FOR

INVESTIGATING THE BIOTRANSFORMATION OF DRUGS.] Pharmazie 1995;50(2):130-8. (Ger)

Rat lymphocyte and mouse myeloma cell cultures were used as in vitro test systems for investigating the biotransformation of drugs. The biochem. properties of both kinds of cells were qual. comparable. No reductive or conjugating activities were present in the cultures. The established and characterized systems were used to study the biotransformation of 4 potential drugs. The Trapidil deriv. AR 12463 (5-piperidino-7-[N-pentyl-N-(beta-hydroxyethyl)]-amino-s-triazolo-[1,5-a]pyrimidine) was transformed into 2 metabolites in both the lymphocyte and myeloma cell cultures. These substances were characterized as the hydroxypentyl- and the hydroxypyrimidine derivs. Both products are the initial metabolites for further degrdn. reactions in vivo in the rat. The immunostimulator AWD 100-041 (3-(2-mercaptoethyl)quinazoline-2,4-(1 H,3H)-dione) was metabolized in both lymphocyte and myeloma cell cultures to the disulfide of the parent compd. After incubation of the S-Me analog of AWD 100-041, itself a metabolite of the drug, sulfoxidized metabolites occurred, which were also detectable in vivo. After incubation of the anticonvulsant AWD 140-076 (4-chlorophenylpyrrole-3-morpholino-2-carboxylic acid Me ester) in the cell cultures 2 metabolites were formed which were oxidized at the morpholine N as well as at the pyrrole skeleton. Both compds. are the main metabolites in metab. in vivo. The biotransformation of the lipoxygenase inhibitor FLM 5011 (2-hydroxy-5-methyllaurophenone oxime) in lymphocyte and myeloma cell cultures was characterized by the formation of the omega-hydroxy deriv. This compd. is the initial metabolite for the further degrdn. of the lauryl side chain. All these substances were tested for cytotoxicity in myeloma cells. The corresponding IC50 values were 4.5 .times. 10-6M for AR 12463, 1.4 .times. 10-5M for AWD 100-041, 1.3 .times. 10-4M for AWD 140-076 and 1.2 .times. 10-4M for FLM 5011. No relationship was found between cytotoxicity and the degree of metab.

39
Kim YS, Park KH. [SCREENING METHOD FOR ANTIHEPATOTOXIC ACTIVITY USING CCl4-INDUCED CYTOTOXICITY IN PRIMARY CULTURED RAT HEPATOCYTES]. Saengyak Hakhoechi 1995;26(1):51-6. (Kor)

To devise an in vitro screening method for antihepatotoxic activity, CCl4-induced cytotoxicities in primary cultured rat hepatocytes were examd. When rat hepatocytes were intoxicated with 0.5, 1.0 or 1.5 mM CCl4 for 1.5, 3 or 19 h, release of LDH, GOT, GPT from hepatocytes was increased in a dose-dependent manner. Treatment with 1.5 mM Ccl4 for 1.5 h showed max. increase in activity of LDH, GOT or GPT released in the medium compared with the control. At this exptl. condition, well known antihepatotoxic substances, glycyrrhizin and silybin markedly inhibited CCl4-induced cytotoxicities. These results demonstrated that the screening method using CCl4-induced injury in primary cultured rat hepatocytes might be suitable in vitro assay for antihepatotoxic activity.

40
Ushimura H, Matsudo Y, Tanaka Y, Tsutsui T. [QUANTITATIVE COMPARISON OF

CYTOTOXICITY OF DENTAL OINTMENTS FOR PERIODONTAL DISEASES TO NORMAL HUMAN
GINGIVAL KERATINOCYTES IN VITRO.] Nippon Shishubyo Gakkai Kaishi 1995;37(1):76-83. (Jpn)

The cytotoxicities of dental ointments utilized for periodontal diseases were examd. with normal human keratinocytes from gingival tissues by the uptake of neutral red (NR assay). The NR assay is a quant. in vitro assay that distinguishes between viable, damaged or dead cells. Cultures from different individuals were established, and secondary cultures in serum-free medium were used. The cytotoxicity of ointments used on mucous membranes in the oral cavity was detd. from the dose-response curves of inhibition of NR uptake in cells treated with ointments for 2 days. As a quant. measure of cytotoxicity, NR 50, i.e. the concn. of ointments that resulted in a 50% decrease in NR uptake relative to untreated controls, was extrapolated from dose-response curves. The rank-order of cytotoxicities (NR 50) of injection type of ointments applied to periodontal pockets was Hinoporon .mchgt. Tetracortisone ointment > Periocline .apprxeq. Terracortrill ointment. The cytotoxicity of Hinoporon was approx. 1300-fold that of Terracortrill ointment. The rank-order of cytotoxicities of spread type of ointments was Dexaltin ointment > Despa KOWA > Tetracycline presteron .apprxeq. Presteron .apprxeq. Kenalog .apprxeq. Aphtasolone. If the same amt. of ointments was applied to the oral mucosa for the same time, the lower-ranking ointments would be less cytotoxicity. These results not only provide useful ests. of relative toxicities of dental ointments to human cells, but also can be useful as a std. for screening of toxicities of the new products.

41
Kaji T, Mishima A, Machida M, Yabusaki K,Suzuki M, Yamamoto C, Fujiwara Y, Sakamoto M, Kozuka H. COMPARATIVE CYTOTOXICITY OF EXOGENOUS CADMIUM-METALLOTHIONEIN AND CADMIUM ION IN CULTURED VASCULAR ENDOTHELIAL
CELLS. Bull Environ Contam Toxicol 1995;54(4):
501-6.

42
Iida T, Tang GQ, Suttikulpitug S, Yamamoto K, Miwatani T, Honda T. ISOLATION OF MUTANT TOXINS OF VIBRIO PARAHAEMOLYTICUS HEMOLYSIN BY IN VITRO MUTAGENESIS.
Toxicon 1995;33(2):209-16.

Thermostable direct hemolysin produced by Vibrio parahaemolyticus is a major virulence factor of the organism. The hemolysin has a variety of biological activities such as lethality to mice, cytotoxicity to cultured cells, cardiotoxicity, and fluid accumulating activity in rabbit ileal loop test. In this study, we attempted to isolate less hemolytic mutant toxins of the thermostable direct hemolysin to use them for analysis of mode of action of the hemolysin. Six mutant toxins were obtained by in vitro mutagenesis of the cloned gene for the hemolysin. Characterization of the mutant toxins demonstrated that single amino acid substitutions at Gly62, Trp65, Thr67, Gly86, Glu116 and Glu138 resulted in a loss or lowering of the hemolytic

activity. Two of the mutant toxins inhibited hemolysis by wild-type toxin on rabbit blood agar plates, while their hemolytic activity was below the detectable level. These mutant toxins would be useful for identifying the as yet unknown receptor for the hemolysin on the target cell membrane.

43
Coldham NG, Moore AS, Dave M, Graham PJ, Sivapathasundaram S, Lake BG, Sauer MJ. [IMIDOCARB RESIDUES IN EDIBLE BOVINE TISSUES AND IN VITRO ASSESSMENT OF IMIDOCARB METABOLISM AND CYTOTOXICITY.] Drug Metab Dispos 1995;23(4):501-5.

Imidocarb residues in liver and muscle were measured by HPLC after a single therapeutic dose to cattle (3 mg imidocarb dipropionate kg-1). Imidocarb and 7-ethoxycoumarin metabolism were compared in three different in vitro systems prepared from bovine liver: cultures of hepatocyte monolayers, precision-cut liver slices, and microsomes. The potential hepatotoxicity of imidocarb residues was tested on hepatocyte monolayers and assessed using the neutral red and lactate dehydrogenase leakage assays. The concentration of imidocarb (mean +/- SD) decreased between days 14 and 224 after treatment from 5.40 +/- 0.61 to 0.12 +/- 0.01 and from 1.05 +/- 0.31 to 0.06 +/- 0.02 microgram g-1 in liver and muscle, respectively. The depletion kinetics of imidocarb fitted a two-compartment model with alpha- and beta-phase half-lives of 31.7 and 48.5 days in liver and 34.9 and 120.7 days in muscle, respectively. Imidocarb metabolites were not detected in any in vitro system. 7-Ethoxycoumarin metabolism was found in all in vitro systems; the predominant metabolite produced by hepatocyte and liver slice cultures was umbelliferone glucuronide. Cytotoxicity of imidocarb (100 microM) to hepatocyte monolayers was maximal after 72 hr treatment and dose-dependent above 10 microM imidocarb. It is most likely that the hepatotoxicity of imidocarb is caused by the parent compound, because no evidence for imidocarb metabolism was found.

44
Flouriot G, Monod G, Valotaire Y, Devaux A, Cravedi JP. XENOBIOTIC METABOLIZING ENZYME ACTIVITIES IN AGGREGATE CULTURE OF RAINBOW TROUT HEPATOCYTES. Seventh International Symposium on Responses of Marine Organisms to Pollutants (Primo 7), Goteborg, Sweden, April 20-22, 1993. Marine Environ Res 1995;39(1-4):293-7.

45
Kleene KC, Wang M, Cutler M, Hall C, Shih D. DEVELOPMENTAL EXPRESSION OF POLY(A) BINDING PROTEIN mRNAs DURING SPERMATOGENESIS IN THE MOUSE. Mol Reprod
Dev 1994;39(4):355-64.

The poly(A) binding protein (PABP), a conserved protein that binds to the 3' poly(A) tail on mRNAs in eukaryotic cells, has been implicated in the regulation of mRNA stability and translation. Two PABP cDNAs with different sequences were isoalted from mouse testis cDNA libraries. The predicted amino acid seuqence of one, PABP1, is nearly identical (98.9%) to human liver PABP, while 80% of the amino acids of the second, PABPt, are identical to mouse and human PABPs. Northern blots reveal that there is one major PABP mRNA species in liver, muscle, kidney, and brain, two in spleen, and at least four in

testis. The levels of PABP mRNA in testis are 5-10-fold higher than in these somatic tissues, but surprisingly the vast majority of all PABP mRNA size variants sediment more slowly than single ribosomes, indicating strong translational repression. Reverse transcriptase-polymerase chain reaction assays demonstrate that PABPt mRNAs are abundant only in testis. Northern blots of RNAs purified from highly enriched spermatogenic cells show that thehigh levels, multiple sizes of PABP mRNAs, and the PABPt mRNA are present in meiotic and early haploid spermatogenic cells, and are sharply reduced in late haploid cells. Comparison of the binding of PABP1 and PABPt to poly(A) Sepharose in vitro revealed subtle differences, even though PABPt contains substitutions for highly conserved arom. amino acids that are thought to be necessary for binding to poly(A). The existence of two PABP isoforms in mouse spermatogenic cells could influence cytoplasmic gene expressino during spermatogenesis.

46
Delmas F, Trocheris V, Murat JC. EXPRESSION OF STRESS PROTEINS IN CULTURED HT29 HUMAN CELL-LINE; A MODEL FOR STUDYING ENVIRONMENTAL AGGRESSION. Int J Biochem Cell Biol 1995;27(4):385-91.

The current study was undertaken to investigate the expression of stress proteins (HSP) in cultured human HT29 cells submitted to stressing events under in vitro conditions. Heat shocks (45~C, for 15-60 min) or cold shocks (+1~C for 4 hr) were found to modify cell growth (growth curves) and to enhance HSP expression. In most cases, changes in HSP expression are much more pronounced than changes in cell growth. Exposure to 8% ethanol for 15 min resulted in both growth inhibition and HSP overexpression. Propanol-1 was found to be more toxic since 5% concentration given for 15 min stops cell growth. 2.5% propanol-1 for 15 min induces a slight reduction of cell growth but a clear-cut overexpression of stress proteins. We conclude that expression of stress proteins, especially those of the HSP68/70 family, constitutes a more sensitive response than changes in growth rate in case of external aggression. This could make our model an interesting biological sensor to environmental physical or chemical pollutants.

47
Schilderman PA, Rhijnsburger E, Zwingmann I, Kleinjans JC. INDUCTION OF OXIDATIVE DNA DAMAGE AND ENHANCEMENT OF CELL PROLIFERATION IN HUMAN LYMPHOCYTES IN VITRO BY BUTYLATED HYDROXYANISOLE. Carcinogenesis 1995;16(3):507-12.

The food additive butylated hydroxyanisole (BHA) has been shown to induce gastrointestinal hyperplasia in rodents by an unknown mechanism. The relevance of this observation for human risk assessment is not clear. We therefore analysed the effect of BHA and its primary metabolites tert-butylhydroquinone (TBHQ) and tert-butylquinone (TBQ) on 8-oxo-deoxyguanosine formation and labelling indices in human lymphocytes in vitro. Analysis of culture medium and cell lysate fractions after administration of BHA or metabolites of BHA revealed that BHA and TBHQ undergo biotransformation in whole blood cultures. Moreover, TBQ can be reduced to TBHQ. While in cultures treated with BHA 50-60% of the dose administered was recovered, a much lower dose recovery was found in cultures treated with either TBHQ or TBQ. This indicates a

considerable binding of these compounds to macromolecules. BHA and TBHQ, as well as TBQ, induced a dose-dependent increase in cell proliferation of phytohaemagglutinin-stimulated lymphocytes, 50 muM being the optimal dose. Since BHA is metabolized to TBHQ, it is not clear which compound is responsible for the proliferation enhancing effects observed in culture. Inhibition of TBHQ metabolism to its semiquinone radical by acetylsalicylic acid (ASA) reduced the increase in labelling indices induced by TBHQ. This indicates that this metabolic pathway is involved in the enhancement of cell proliferation induced by the hydroquinone. HPLC-ECD analysis of oxidative DNA damage in lymphocytes exposed to 10, 50 and 100 muM BHA, TBHQ or TBQ respectively showed that BHA was not capable of inducing oxidative DNA damage to a significant degree. TBQ and, in particular, TBHQ at a dose of 50 muM (the optimal dose for induction of cell proliferation), however, increased lymphocyte 7-hydroxy-8-oxo-2'-deoxyguanosine formation by 320 and 680% respectively. Inhibition of prostaglandin H synthase by ASA in cultures treated with TBHQ decreased the oxidation ratio significantly, confirming the significance of this enzyme system in the mechanism of toxicity of BHA.

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Hilton J, Kimber I. THE MURINE LOCAL LYMPH NODE ASSAY. Methods Mol Biol 1995;43:227-35.

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Gillies GE, Buckingham JC. THE APPLICATION OF IN VITRO MODELS OF HYPOTHALAMIC FUNCTION IN TOXICITY TESTING. Methods Mol Biol 1995;43: 95-107.

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Gillies GE, Buckingham JC. THE APPLICATION OF IN VITRO MODELS OF ANTERIOR PITUITARY FUNCTION IN TOXICITY TESTING. Methods Mol Biol 1995;43:81-93.

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Hodgson E. CHEMICAL AND ENVIRONMENTAL FACTORS AFFECTING METABOLISM OF XENOBIOTICS. In: Hodgson E and Levi PE, editors. Introduction to Biochemical Toxicology. 2nd ed. East Norwalk (CT): Appleton and Lange; 1994. p. 153-75.

52
Jiang Y, Moller G. IN VITRO EFFECTS OF HGCl2 ON MURINE LYMPHOCYTES: I. PREFERABLE ACTIVATION OF CD4+ T CELLS IN A RESPONDER STRAIN. J Immunol 1995;154(7):3138-46.

Mercury-induced autoimmune disorders have been demonstrated in rats and mice injected with HgCl2. We have studied the ability of HgCl2 to activate murine lymphocytes in vitro and found that it induced increased DNA synthesis, which peaked at days 4 to 6. Other metal ions, such as Mg2+ and Zn2+, had no or much less effect. Consistent with the in vivo studies, there were strain differences, and the most significant increase in thymidine uptake was induced in A.SW and BALB/c spleen cells. Both T lymphocytes and adherent cells were required for activation, and anti-CD4 Ab completely abrogated HgCl2-induced proliferation, suggesting the involvement of T CD4+ and CD8+ T cells from BALB/c mice (responder strain). In contrast, only CD8+ T cells from the nonresponder DBA/2 mice were transformed. These findings indicate that helper

T cells play a crucial role for the immunologic effects caused by HgCl2 and determine the ability of different mouse strains to respond to HgCl2.

53
Hwang DF, Lin JF, Jengs SS. STUDIES ON SUSPENDING CONDITIONS FOR ISOLATED EEL HEPATOCYTES. J Fish Soc Taiwan 1994;21(3):273-80.

To establish fish hepatocytes as experimental materials for in vitro system of toxicological and metabolic studies, the livers of Japanese eel Auguilla japonica were used to isolate hepatocytes by using perfusion method. Based on the viability of hepatocytes by staining with trypan blue the optimal suspending conditions for eel hepatocytes were determined. It was found that the optimal suspending temperature, time, cell concentration and pH value were 37~ C, 2 hr, 207 cells/ml and 7.5, respectively. It was also found that the viability of isolated eel hepatocytes was improved when the suspending buffer was added with either 5.6 mM glucose or 2.5 mM Ca++.

54
Kristen U, Kappler R. THE POLLEN TUBE GROWTH TEST. Methods Mol Biol 1995;43:189-98.

55
Blein-Sella O, Adolphe M. RABBIT ARTICULAR CHONDROCYTE FUNCTIONAL TOXICITY TEST. Methods Mol Biol 1995;43:169-75.

56
Bidey SP. THYROID FOLLICULAR CELLS IN MONOLAYER CULTURE IN VITRO MODELS FOR THYROID TOXICITY TESTING. Methods Mol Biol 1995;43:33-42.

57
Becerro MA, Uriz MJ, Turon X. MEASURING TOXICITY IN MARINE ENVIRONMENTS: CRITICAL APPRAISAL OF THREE COMMONLY USED METHODS. Experientia 1995;51(4):414-8.

Toxicity quantification is important in environmental monitoring, in the field of natural products, and in chemical ecology. The sensitivity and precision of three commonly used methods detecting toxicity in marine environments were compared, using the toxic marine sponge Crambe crambe as a test organism. The paper disk diffusion method (run with marine bacteria) showed the least sensitivity and did not permit toxicity levels to be quantified. The sea urchin and the MICROTOX tests showed greater sensitivity, and the latter had the higher precision. The relative performance of these methods is discussed. It is concluded that the MICROTOX bioassay displays the best characteristics for toxicity quantification.

58
Blanquart C, Giuliani I, Houcine O, Jeulin C, Guennou C, Marano F. IN VITRO EXPOSURE OF RABBIT TRACHEAL EPITHELIUM TO SO2: EFFECTS ON MORPHOLOGY AND
CILIARY BEATING. Toxicol In Vitro 1995;9(2):123-32.

The aim of this in vitro study was to characterize the direct effects of short-term exposure to low concentrations of sulfur dioxide (SO2) on both the morphology and the physiology of rabbit tracheal primary cultures. Scanning electron microscopy (SEM) studies revealed that ciliated cells exposed for 1 hr to 10 ppm or 30 ppm SO2 exhibited aggregated cilia. Transmission electron microscopy revealed numerous swollen mitochondria in cells exposed to 30 ppm SO2 for 1 hr. This morphological damage to cells was coupled with physiological alterations. A 25% decrease in ciliary beat frequency (CBF) was measured in cells exposed to 30 ppm SO2. This inhibition was partially reversible within 24 hr. This SO2 concentration also induced a significant depletion of cellular ATP content which was completely restored after a 24-hr recovery period. A correlation was found between cellular ATP level depletion and CBF decrease.

59
Krautschick I, Krugmann J, Neuenfeld M. THE EFFECT OF PEROXIDES ON THE VASCULAR ENDOTHELIUM OF ISOLATED PIG AORTA IN VITRO. Exp Toxicol Pathol 1995;47(1):51-61.

The effect of peroxide on endothelial cells (perfused pig aorta) was examined using an in vitro perfusion model. Hydrogen peroxide was added to the perfusion medium (pig serum together with a buffer solution) which was expected to lead to an increased oxidation of lipids and lipoproteins. Oxidation processes of this type play a decisive role in the pathogenesis and progression of arteriosclerosis. The aim of the present investigation was to demonstrate by introducing hydrogen peroxide (H2O2) in varying concentrations (0.5-1.5%), the destructive impact of peroxides on the endothelium, while these cells are believed to play a key role in the pathogenesis of arteriosclerosis. The extent of endothelial cell impairment was assessed by means of silver staining visualization of endothelial cell borders as well as light- and scanning-electronmicroscopic investigation. It was discovered that the endothelial cells show increasing impairment after 10 h of perfusion due to the effect of peroxide (hydrogen peroxide).

60
Argese E, Bettiol C, Ghelli A, Todeschini R, Miana P. SUBMITOCHONDRIAL PARTICLES AS TOXICITY BIOSENSORS OF CHLOROPHENOLS. Environ Toxicol Chem 1995;14(3):363-8.

An in vitro bioassay procedure was used to investigate the toxic action of chlorophenols on mitochondrial respiratory parameters. The toxicity of these compounds was evaluated by determining their effects on the energy-coupled reverse electron transfer (RET) in submitochondrial particles (SMPs) from beef heart mitochondria. The bioassay procedure is based on the spectrophotometric recording of the effects of toxicants on the rate of NAD+ reduction, induced by ATP and succinate at the first site level of the respiratory chain. The toxicity end point was expressed as the toxicant concentration that causes 50% inhibition of NAD+ reduction rate (EC50). The EC50 values determined for the 14 tested chlorophenols ranged from 17 mg/L for 2-chlorophenol to 0.081 mg/L for pentachlorophenol, indicating a general trend of increasing toxicity with increasing chlorine substitution. Among chlorophenol isomers, which have

the same number of chlorine atoms, a lesser toxicity was associated with ortho-substituted chlorophenols, whereas meta-substituted chlorophenols were much more toxic. The EC50 values were compared with the toxicity data for a variety of bioassays, by means of linear regression analysis. High degrees of correlation obtained with toxicity tests involving different freshwater species demonstrate the ability of SMPs to reproduce the toxic effects of the tested compounds upon aquatic organisms. This supports the assessment that the respiratory chain is the main target of this class of toxicants. Results obtained with chlorophenols and, in previous studies, with other environmental contaminants confirm the suitability of the SMP bioassay as a prescreening or complementary short-term test for monitoring aquatic toxicity.

61
Celander M, Broman D, Forlin L, Naf C. EFFECTS OF PETROLEUM HYDROCARBONS ON THE HEPATIC CYTOCHROME P450 1A1 SYSTEM IN RAINBOW TROUT. Seventh International Symposium on Responses of Marine Organisms to Pollutants (Primo 7), Goteborg, Sweden, April 20-22, 1993. Marine Environ Res 1995;39(1-4):61-5.

Burke MD, Brown D, Mayer RT, Houlihan DF. ALKOXYQUINOLINE O-DEALKYLATION A NEW FLUORIMETRIC ASSAY FOR HYDROCARBON-INDUCED CYTOCHROME P450 IN FISH. Seventh
International Symposium on Responses of Marine Organisms to Pollutants (Primo 7), Goteborg, Sweden, April 20-22, 1993. Marine Environ Res 1995;39(1-4):57-60.

62
Bylander JE, Li SL, Sens MA, Sens DA. EXPOSURE OF HUMAN PROXIMAL TUBULE CELLS TO CYTOTOXIC LEVELS OF CdCl2 INDUCES THE ADDITIONAL EXPRESSION OF METALLOTHIONEIN 1A mRNA. Toxicol Lett 1995;76(3):208-17.

Humans, in contrast to animals, have a complex expression of metallothionein (MT) genes which involves many MT isoforms encoded by a family of genes containing an upper limit of 12 possible functional genes. It is unknown if these human isoforms of MT have distinct functions or if they simply represent a non-essential duplication of gene function. In the present study, MT protein and mRNA for the MT-2A, MT-1A, B, E, F, and G genes was determined for 3 isolates of human proximal tubule (HPT) cells having distinct sensitivities to cadmium. For all 3 HPT isolates, the expression of MT protein and mRNA for the MT-2A, MT-1E, MT-1F and MT-1G isoforms was similar among the isolates and demonstrated no correlation to lethality. However, each isoform mRNA was expressed at different levels when compared to one another. In contrast, the expression of MT-1A mRNA differed in expression and correlated with the differing lethalities displayed by each isolate. The finding of different profiles of mRNA expression provides evidence that the MT isoforms may have unique functions and that mRNA for the MT-1A gene could be a potential marker for heavy metal exposure and/or toxicity.

63
Champion AR, Hanson JA, Court JB, Venables SE. THE MICRONUCLEUS ASSAY: AN EVALUATION OF ITS USE IN DETERMINING RADIOSENSITIVITY IN VITRO. Mutagenesis

1995;10(3):203-8.

The cytokinesis-block micronucleus assay was used to measure radiosensitivity in vitro in a panel of seven cell lines. Six of these cell lines were used to study the major parameters of this assay. We observed varying sensitivities following cytochalasin-B exposure. Treatment with 1 mug/ml cytochalasin-B for 24 h reduced cell survival in four of the six cell lines by > 60%. Cytochalasin-B concentration and post-irradiation culture time were both found to influence cell-response. In three cell lines (V39, V134 and HX142), a decrease in cytochalasin-B concentration (2-0.5 mug/ml) resulted in an increase in the frequency of radiation-induced micronuclei per binucleate cell. In other cell lines, either the opposite (V7M, CHO-K1) or no effect (WiDr) was seen. A linear dose-response was observed between induced damage expressed as the frequency of micronuclei and radiation dose in all but one melanoma (V39) cell line. Evidence for radiation-induced division-delay, with the maximum frequency of binucleation in irradiated cultures occurring 24-48

h after that of controls, was only seen in two cell lines. Of particular note, and in contrast to some other published reports, was the lack of a general correlation between cell-response measured in the clonogenic and the cytokinesis-block micronucleus assays. Consideration of lethal lesions, determined from the clonogenic dose-response curve, with respect to micronucleus frequency showed a complex relationship, with one micronucleus per binucleate cell corresponding to a wide range of lethal lesions depending on the cell line. It has been postulated that the binucleate cell with no micronuclei may represent the surviving cell; however, we found no correlation between the slope of the frequency of these cells with respect to radiation dose and the clonogenic alpha slope. These observations should be considered prior to attempting to use the cytokinesis-block micronucleus assay to measure in vitro radiosensitivity in human tumour cells.

64
Clare C. MUTATION ASSAYS IN BACTERIA. Methods Mol Biol 1995;43:297-306.

65
Dean S. MEASUREMENT OF UNSCHEDULED DNA SYNTHESIS IN VITRO USING PRIMARY RAT
HEPATOCYTE CULTURES. Methods Mol Biol 1995;43: 267-76.

66
Bryan FL. PROCEDURES TO USE DURING OUTBREAKS OF FOOD-BORNE DISEASE. In: Murray
PR, et al, editors. Manual of Clinical Microbiology. 6th ed. Washington DC:
American Society for Microbiology; 1995: p. 209-26.

67
Edwards AJ, Anderson D, Phillips BJ. INDUCTION OF POLYPLOIDY IN HUMAN LYMPHOCYTES IN VITRO BY EXCESS ADENINE, BUT NOT BY ADENOSINE. Environ Mol Mutagen 1995;25(3):197-201.

It is known that high levels of DNA precursors can be both clastogenic and

mutagenic in cultured cell lines and in vivo. The purpose of the present study was to examine at an observational level the cytogenetic effects of adenine and adenosine in primary human cell cultures. Human peripheral blood lymphocytes from four donors were cultured and treated with a range of concentrations of adenine and adenosine. Although no increase in sister chromatid exchange (SCE) frequency was observed with either compound, there was a statistically significant, dose-related increase in the proportion of polyploid cells in cultures treated with adenine, but not in those treated with adenosine. Some of the polyploid metaphases found after adenine treatment contained diplochromosomes, suggesting that endoreduplicotion might have been involved in polyploid formation in these cells. It is concluded that a high level of adenine can cause genetic changes in human lymphocytes by interfering with mitosis, perhaps by disturbing the balance of DNA precursor pools.

68
Dinjus U, Haenel I. CULTIVATION OF SALMONELLA IN CONTACT WITH EPITHELIAL CELLS. Microbiol Res 1995;150(1):99-102.

An in vitro cultivation model for Salmonella having contact to epithelial cells was developed, which led to an increase in the production of toxic substances. The toxin assay on CHO-K1 cells was used for the determination of the toxic activities. Salmonella strains cultivated in contact with a monolayer of the intestinal cell line IEC-6 produced considerably more toxin than Salmonella strains cultivated on VERO cells. The toxin formed was heat-labile.

69
Falk PM, Sabater RT, Carballo DD Jr. RESPONSE OF THE HUMAN HEMATIC TISSUE CULTURES HEP-G2 AND WRL-68 TO COCAINE. J Pharmacol Toxicol Methods 1995;33(2):113-20.

The hydrolytic metabolism of cocaine into benzoylecgonine, ecgonine methyl ester, and ecgonine was studied in the human hepatoma cell line Hep-G2 and in the nontumorigenic fetal hepatic cell line WRL-68. Also, the toxicological response of these cells to cocaine was compared to previously published results obtained with perfused liver cells and in vivo systems. Our experiments indicated that Hep-G2 appear to have similar metabolic and toxicological patterns to in vivo and perfused cell systems. The WRL-68 tissue culture system was found to be less similar. These results suggest Hep-G2 cells can be utilized to study cocaine metabolism and toxicology, and possibly in studies involving other xenobiotic compounds.

70
Fiskesjo G. ALLIUM TEST. Methods Mol Biol 1995;43:119-27.

71
Male KB, Brown RS, Luong J HT. ENZYMATIC OXIDATION OF WATER-SOLUBLE CYCLODEXTRIN-POLYNUCLEAR AROMATIC HYDROCARBON INCLUSION COMPLEXES, USING
LIGNIN PEROXIDASE. Enzyme Microb Technol 1995;17(7):607-14.

alpha-, beta-, gamma-, and 2-hydroxypropyl-beta-cyclodextrins were capable of forming water-soluble inclusion complexes with several polynuclear aromatic hydrocarbons (PAHs). The highest solubilities were noted for beta-cyclodextrin and 2-hydroxypropyl-beta-cyclodextrin (hpbetaCD). The solubility of PAHs in hpbetaCD was enhanced 224-fold and 7,500fold for naphthalene and benzo(a)pyrene, respectively, with other PAHs yielding values between these limits. The ability of lignin peroxidase (LiP) to cyclodextrin-included substrates was similar to that previously reported for mixed solvent systems. The enzyme oxidized anthracene, pyrene, and benzo(a)pyrene but not naphthalene, phenanthrene, chrysene, and benzo(e)pyrene. The lignin peroxidase exhibited a preference for oxidizing either anthracene or benzo(a)pyrene when mixed with pyrene. On the basis of fluorescence measurement, anthracene and benzo(a)pyrene were easily distinguished by exciting at 250 nm for anthracene and 295 nm for benzo(a)pyrene. Veratryl alcohol severely inhibited the pyrene assay, with 50% inhibition noted at 0.3 mm while veratryl alcohol activated the reactions between LiP and either anthracene or benzo(a)pyrene. Maximal activation was obtained at 1.5 mm veratryl alcohol and no inhibitory effect was detected up to 4.0 mm. Under identical conditions, the rate of reaction with veratryl alcohol (4.0 mm) was 11- and 14-fold faster for benzo(a)pyrene and anthracene, respectively, when compared to the assays in the absence of veratryl alcohol.

72
Fisher RL, Hasal SJ, Sipes IG, Gandolfi AJ, Brendel K. COMPARATIVE METABOLISM AND TOXICITY OF DICHLOROBENZENES IN SPRAGUE-DAWLEY, FISCHER-344 AND HUMAN
LIVER SLICES. Hum Exp Toxicol 1995;14(5):414-21.

1 Precision-cut liver slices, prepared from Sprague-Dawley and Fischer-344 rats and donated human liver tissue, were used to identify differences in 1,2-dichlorobenzene (1,2-DCB), 1,3-dichlorobenzene (1,3-DCB) and 1,4-dichlorobenzene (1,4-DCB) metabolism and how it may relate to toxicity. 2 Rat and human liver slices were incubated with 1 mM of either dichlorobenzene to determine metabolism and toxicity, at 2 and 6 h of organ culture. 3 The human liver slices metabolised the dichlorobenzenes to a greater extent than those from either of the rat strains. Liver slices from the Fischer-344 strain had a higher metabolic rate than the slices from the Sprague-Dawley rat strain. 4 The metabolic rate of dichlorobenzene isomers did not consistently correlate with its toxicity. For example, human slices did not exhibit any hepatotoxicity, even though they metabolised these compounds to a greater extent than either rat strain. 5 Cross species covalent binding did not correlate with toxicity endpoints measured in this study. 6 The phase two metabolite profiles for each of the isomers in human and rat slices were similar in that the glutathione-cysteine conjugate was the major metabolite. 7 The use of an in vitro system which utilises human liver slices might provide an important bridge between animal derived data and the human situation.

73
Na MR, Koo SK, Kim DY, Park SD, Rhee SK, Kang KW, Joe CO. IN VITRO INHIBITION OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION BY CHEMICAL CARCINOGENS. Toxicology 1995;98(1-3):199-206.

This study was conducted to assess the effects of chemical carcinogens on the gap junction-mediated intercellular communication in cultured mammalian cells. The method of scrape-loading dye transfer of lucifer yellow was adapted as a measure of gap junctional communication. Clone 9 cells derived from rat liver were treated with a model chemical carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and the gap junctional communication was assessed by measuring the transfer of scrape-loaded lucifer yellow dye. When cells were treated with the carcinogen at 0.3 mg/ml, the fluorescent dye transfer was inhibited by 90% in 60 min. Other chemical agents, which include direct or indirect carcinogens and antitumor drugs, were also examined for their effects on the gap junctional communication. Direct carcinogens, such as MNNG, hydroxylamine and ethidium bromide, exhibited strong inhibition of intercellular communication, while indirect carcinogens, such as aflatoxin B1 and ethionine, exerted minor effects. Effects of test chemicals on the cell communication through gap junctions were readily quantitated by counting the number of cells stained with the fluorescent dye.

74
Naughton BA, Sibanda B, San Roman J, Naughton GK. CHARACTERIZATION AND USE OF LONG-TERM LIVER CULTURES TO EVALUATE THE TOXICITY OF CYCLOPHOSPHAMIDE OR
BENZENE TO BONE MARROW CULTURES. In: Reinhardt CA, editor. Alternatives to Animal Testing: New Ways in the Biomedical Sciences, Trends and Progress; Symposium; 1992 Nov 30; Zurich, Switzerland. New York: VCH Publishers, Inc: 1994. p. 147-57.

Flint O. A TIMETABLE FOR REPLACING REDUCING AND REFINING ANIMAL USE WITH THE HELP OF IN VITRO TESTS THE LIMULUS AMOEBOCYTE LYSATE TEST LAL AS AN EXAMPLE.
In: Reinhardt, CA, editor. Alternatives to Animal Testing: New Ways in the Biomedical Sciences, Trends and Progress; Symposium; 1992 Nov 30; Zurich, Switzerland. New York: VCH Publishers, Inc; 1994: p. 27-43.

75
Berg OH, Henriksen RN, Steinsvag SK. THE EFFECT OF A BENZALKONIUM CHLORIDE-CONTAINING NASAL SPRAY ON HUMAN RESPIRATORY MUCOSA IN VITRO AS A
FUNCTION OF CONCENTRATION AND TIME OF ACTION. Pharmacol Toxicol 1995;76(4):245-9.

Human respiratory mucosa was exposed to oxymetazoline nasal spray in varying concentrations and for varying periods of time in vitro. The drug destroyed the tissue in a concentration- and time-dependent manner. In the experiments with various concentrations of the spray, some tissue fragments retained their viability throughout the experiment. This number increased parallel to a decrease in concentrations of the test substance. All the tissue fragments exposed to undiluted nose spray underwent severe destructive alterations during the exposure period. These alterations appeared first and were most extensive in those exposed for the longest periods of time. It has previously

been demonstrated that the toxic effect of oxymetazoline nasal spray in vitro is probably due to the preservative benzalkonium chloride. The apparent lack of consistency between the toxic effects of benzalkonium chloride in vitro and in vivo is discussed, with special reference to protective systems absent in vitro but present in vivo.

76
Potgieter FJ, Torronen R, Wilke PI. THE IN VITRO ENZYME-INDUCING AND CYTOTOXIC PROPERTIES OF SOUTH AFRICAN LABORATORY ANIMAL CONTACT BEDDING AND NESTING
MATERIALS. Lab Anim 1995;29(2):163-71.

Enzyme-inducing and cytotoxic effects of South African bedding materials were investigated using a mouse hepatoma cell line, Hepa-1, cell culture system. This cell culture system is a convenient and sensitive method for the screening of bedding materials for the presence of compounds that could be potentially harmful to animals and thus the experimental outcome. Cells were exposed to acetone extracts of the different materials or their components. Corn cobs displayed very little or no CYP1A1-inducing or cytotoxic effects, whilst vermiculite and unbleached pulp from pine and eucalyptus showed greater induction and cytotoxic properties. The latter properties were lower than those produced by the different recycled paper extracts. Pine shavings (Pinus elliottii) and the different wood components making up industrial sawdust expressed the highest cytotoxic and CYP1A1-inducing properties.

77
Jontell M, Hanks CT, Bratel J, Bergenholtz G. EFFECTS OF UNPOLYMERIZED RESIN COMPONENTS ON THE FUNCTION OF ACCESSORY CELLS DERIVED FROM THE RAT INCISOR
PULP. J Dent Res 1995;74(5):1162-7.

Monomeric resin components from dental composites are toxic to fibroblasts in culture and thus may interfere with the local immune system of the pulp, reducing its effective defense potential, either by cytotoxicity or by a more specific immune mechanism. Therefore, the present study was undertaken to observe the cytotoxic effects elicited by certain unpolymerized components of resin composites upon the function of accessory pulp cells in mitogen-induced proliferation of T-lymphocytes. Accessory cells from the rat incisor pulp were released following enzymatic digestion with collagenase. The assay included incubation of these cells with purified T-lymphocytes from cervical lymph nodes for 72 h in the presence of different concentrations of the resin components. The proliferative T-lymphocyte response was monitored by 3H-thymidine incorporation. Initially, we conducted experiments on spleen cells to determine the proper concentration intervals for suitable testing of the resin components. To assess the individual susceptibility of accessory cells and T-lymphocytes, we pre-treated each of these cells with some of the test materials prior to assay. At low concentrations, urethane dimethacrylate (UDMA), bisglycidyl methacrylate (bis-GMA), triethylene glycol dimethacrylate (TEGDMA), and bis-phenol A (BPA) increased spleen cell proliferation to concanavalin A (con A). Purified T-lymphocytes stimulated by

pulpal cells did not show enhanced responses to UDMA, bis-GMA, glycidyl

mehtacrylate (GMA), or N,N,-dihydroxyethyl-p-toluidine (DHEpT). At higher concentrations, all substances except camphoroquinone (CAMP) showed inhibitory effects in both test systems. The in vitro study shows that resin components can evoke either immunosuppression or immunostimulation on mitogen-driven proliferation of purified T-lyumphocytes and spleen cells.

78
Chiao C, Zhang Y, Kaufman DG, Kaufmann WK. DERIVATION OF PHENOBARBITAL-RESPONSIVE IMMORTAL RAT HEPATOCYTES. Am J Pathol 1995;146(5):1248-59.

Two lines of rat hepatocytes, designated 6/15 and 6/27, were obtained from carcinogen-treated livers by cultivation in medium containing the liver tumor promoter, phenobarbital (PB). Both lines appeared to be PB-responsive and to have an unlimited in vitro proliferative lifespan, i.e., immortality. The ability of pure 6/27 hepatocytes to form colonies from single cells was strictly dependent upon PB; it was reduced by 97 to 99% in the absence of PB. These hepatocytes were not tumorigenic. For 6/27 hepatocytes in early passages where cultures contained fibroblast contaminants and later when they were a pure culture, PB was able to enhance colony growth from single cells and facilitated population expansion by sustaining DNA synthesis and by inhibiting cell lysis. The 6/15 line displayed PB-dependent colony formation and was not tumorigenic at early passages. At later passages 6/15 hepatocytes were less dependent on PB for colony formation, and they formed hepatocellular carcinoma when transplanted into livers of syngeneic rats. The demonstration that PB sustained the proliferation and viability of hepatocytes with enhanced growth capacity and indefinite proliferative lifespan suggests that PB may be necessary for progression of these chemically initiated hepatocytes to immortal and tumorigenic lines in vitro.

79
Pelin K, Kivipensas P, Linnainmaa K. EFFECTS OF ASBESTOS AND MAN-MADE VITREOUS FIBERS ON CELL DIVISION IN CULTURED HUMAN MESOTHELIAL CELLS IN COMPARISON TO
RODENT CELLS. Environ Mol Mutagen 1995; 25(2):118-25.

We report the effects of chrysotile and crocidolite asbestos, and glass and rock wool fibers (man-made vitreous fibers, MMVF) on the duction of binucleate cells in vitro. The response of human mesothelial cells (target cells in fiber carcinogenesis) and rodent cells was compared. Human primary mesothelial cells, MeT-5A cells (an immortalized human mesothelial cell line), and rat liver epithelial (RLE) cells were exposed to asbestos and MMVF samples of

similar size range. Milled glass wool, milled rock wool, and titanium dioxide were used as non-fibrous particle controls. All four fiber types caused statistically significant increases in the amount of binucleate cells in human primary mesothelial cells and MeT-5A cells (in the dose range 0.5-5.0 micrograms/cm2). Chrysotile and crocidolite asbestos were more effective (1.3-3.0-fold increases) than thin glass wool and thin rock wool fibers

(1.3-2.2-fold increases). However, when the fiber doses were expressed as the number of fibers per culture area, the asbestos and MMVF appeared equally effective in human mesothelial cells. In RLE cells, chrysotile was the most potent inducer of binucleation (2.9-5.0-fold increases), but the response of the RLE cells to crocidolite, thin glass wool, and thin rock wool fibers was similar to the response of the human mesothelial cells. No statistically significant increases in the number of bi- or multinucleate cells were observed in human primary mesothelial cells or RLE cells exposed to the non-fibrous dusts. In MeT-5A cells exposed to 5 micrograms/cm2 of milled glass wool and milled rock wool, as well as in cultures exposed to 2 and 5 micrograms/cm2 of TiO2, significant increases were, however, observed. Our results show that rodent cells respond differently to mineral fibers than human cells. The results also add evidence to the suggested importance of disturbed cell division in fiber carcinogenesis.

80
Rat P, Korwin-Zmijowska C, Warnet JM, Adolphe M. NEW IN VITRO FLUORIMETRIC MICROTITRATION ASSAYS FOR TOXICOLOGICAL SCREENING OF DRUGS. Cell Biol Toxicol 1994;10(5-6):329-37.

Flow cytometry has been widely used to quantify fluorescent probes in cell culture. However, FCM is not adapted to toxicological screenings due to the cost, the length and the poor reproducibility of this technique. Moreover, several multicenter studies have preferred microtitration methodologies for drug screening. A new fluorimetric technology has been designed that is sensitive and adapted to direct screening in 96-well microplates. This fluorimeter uses cold light technology (CLF) with chemical and physical modifications of the lighting system (Rat et al., 1995). CLF allows reading of UV, visible and near infrared fluorescence by increasing light energy (from 1000 to 2300 lumens) and reducing the calorific part of light (IR > 900 nm, Joule effect). It induces a decrease in background and a 500- to 1000-fold improvement of detection limit of probes in comparison with classical fluorimeters and permits detection of pg/ml to fg/ml. CLF allows easy evaluation of cell injury induced by physical agents (UVA) or chemical toxins (CCl4). Four biological endpoints for cytotoxicity evaluation have been tested with several probes: proliferation (H33258); viability (fluorescent Neutral Red); cell-cell adhesion (calcein-AM); and mitochondrial metabolic effects (Rhodamine 123). Rh123 assay appeared more sensitive than fluorimetric or photometric detection of Neutral Red assay. Cold light fluorimetry (CLF) permits direct detection of low concentrations of probes (pg/ml to fg/ml). CLF is shown to improve classical cytotoxicity assays and, owing to its adaptability to microtitration (in 6-, 12- or 96-well plates and in Petri

dishes), it is thus a promising alternative to flow cytometry for drug cytotoxicity screening.

81
Ruotsalainen M, Savolainen KM. EFFECTS OF A PROTEIN KINASE C INHIBITOR, Ro 31-7549, ON THE ACTIVATION OF HUMAN LEUKOCYTES BY PARTICULATE STIMULI. Hum Exp Toxicol 1995;14(3):266-72.

1. A new specific protein kinase C (PKC) inhibitor, Ro 31-7549, was used to explore the mechanisms by which particulate stimuli, quartz and chrysotile, stimulate human polymorphonuclear leukocytes (PMNL) to produce reactive oxygen metabolites (ROM). Also soluble stimuli, formyl-Methionyl-Leucyl-Phenylalanine (fMLP) and phorbol myristate acetate (PMA) were used. 2. Ro 31-7549 inhibited chrysotile-induced free intracellular calcium ((Ca2+)i) elevations but did not have an effect on quartz-induced elevations of (Ca2+)i. Both quartz and chrysotile induced production of ROM were partially inhibited by Ro 31-7549. fMLP-induced elevation of (Ca2+)i was inhibited by Ro 31-7549 whereas PMA did not affect (Ca2+)i. Ro 31-7549 strongly inhibited fMLP-induced ROM production, and completely abolished that induced by PMA. 3. These results suggest that PKC may have an important role in the activation of PMNL to produce ROM by particulate and soluble stimuli. However, the inhibition of chrysotile-, but not of quartz-induced (Ca2+)i elevations by Ro 31-7549 provides evidence that both PKC-dependent and -independent mechanisms may play a role in the activation of human leukocytes to produce ROM.

82
Ogawa N, Hirose T, Fukushima K, Suwa T, Satoh T. METABOLISM OF A NITRATE ESTER, DIHYDROPYRIDINE DERIVATIVE IN RABBIT HEPATIC MICROSOMES AND CYTOSOL.
Xenobiotica 1995;25(3):283-90.

1. The metabolism of a nitrate ester-substituted dihydropyridine derivative (NND) in vitro was characterized with rabbit hepatic microsomes and cytosol. 2. Denitration activity was located in both the microsomal and cytosolic fractions, whereas oxidation to the pyridine analogue was solely located in the microsomal fraction. 3. Oxidation to the pyridine analogue required NADPH and was inhibited by carbon monoxide, miconazole and SKF-525A, suggesting that oxidation was catalysed by P450. 4. Denitration activity in the microsomes required either NADPH or GSH. Together with these results, responses to various inhibitors indicate participation of both P450 and glutathione S-transferase (GST). 5. Denitration activity in cytosol was activated by glutathione (GSH), and by dithiothreitol (DTT) to a greater extent. GSH-dependent denitration was inhibited by S-hexyl GSH, an inhibitor of GST, but DTT-dependent denitration was not. Moreover, the formation patterns of the mono-denitrated metabolites, M1 and M2, were shown to be different in each incubation condition. 6. These results suggest that the denitration of NND in cytosol could be catalysed by a GSH-independent enzyme as well as the GSH-dependent enzyme, GST.

83
Link CJ Jr, Evans MK, Cook JA, Muldoon R, Stevnsner T, Bohr VA. CAFFEINE INHIBITS GENE-SPECIFIC REPAIR OF UV-INDUCED DNA DAMAGE IN HAMSTER CELLS AND IN
HUMAN XERODERMA PIGMENTOSUM GROUP C CELLS. Carcinogenesis 1995;16(5):1149-55.

We have studied the effect of caffeine on gene- and strand-specific DNA repair after exposure of Chinese hamster ovary cells and human xeroderma pigmentosum

complementation group C (XPC) cells to ultraviolet irradiation (UV). In hamster cells, caffeine inhibited the repair of cyclobutane dimers (CPDs) in the dihydrofolate reductase (DHFR) gene by up to 66% after 8 h of repair incubation. This effect was dose-dependent, with more inhibition at 10 than at 1.5 mM caffeine. The inhibition was due to decreased repair in the transcribed strand of the hamster DHFR gene. This decrease in repair of CPDs in the DHFR gene correlated with an enhancement of UV-induced cell killing by caffeine. DNA repair was also measured in the overall genome by repair-replication analysis. In hamster cells, caffeine caused a modest enhancement of repair. Caffeine did not produce a significant effect on cell cycle progression up to 8 h after UV irradiation, but it caused a distinct block in early S phase during the 24 h post-irradiation period. In XPC cells, 10 mM caffeine inhibited the removal of CPDs from the transcribed strand of the DHFR gene by 92%. The removal of all photoproducts from the overall genome was inhibited by 26% in these cells. Since the residual repair in XPC cells is thought to occur in active genomic regions, we propose that caffeine preferentially inhibits gene-specific repair.

84
Zastawny TH, Altman SA, Randers-Eichhorn L, Madurawe R, Lumpkin JA, Dizdaroglu M, Rao G. DNA BASE MODIFICATIONS AND MEMBRANE DAMAGE IN CULTURED MAMMALIAN CELLS TREATED WITH IRON IONS. Free Radic Biol Med 1995;18(6):1013-22.

We investigated DNA base damage in mammalian cells exposed to exogenous iron ions in culture. Murine hybridoma cells were treated with Fe(II) ions at concentrations of 10 muM, 100 muM, and 1 mM. Chromatin was isolated from treated and control cells and analyzed by gas chromatography/mass spectrometry for DNA base damage. Ten modified DNA bases were identified in both Fe(II)-treated and control cells. The quantification of modified bases was achieved by isotope-dilution mass spectrometry. In Fe(II)-treated cells, the amounts of modified bases were increased significantly above the background levels found in control cells. Dimethyl sulfoxide at concentrations up to 1 M in the culture medium did not significantly inhibit the formation of modified DNA bases. A mathematical simulation used to evaluate the plausibility of DNA damage upon Fe(II) treatment predicted a dose-dependent response, which agreed with the experimental results. In addition, Fe(II) treatment of cells increased the cell membrane permeability and caused production of lipid peroxides. The nature of DNA base lesions suggests the involvement of the hydroxyl radical in their formation. The failure of dimethyl sulfoxide to inhibit their formation indicates a site-specific mechanism for DNA damage with involvement of DNA-bound metal ions. Fe(II) treatment of cells may increase the intracellular iron ion concentration and/or cause oxidative stress releasing metal ions from their storage sites with subsequent binding to DNA. Identified DNA base lesions may be promutagenic and play a role in pathologic processes associated with iron ions.

85
Warren WG. ESTIMATION WITH VARYING DETECTION LIMITS. In: Markert B, editor. Environmental Sampling for Trace Analysis. New York: VCH Publishers, Inc: 1994. p. 109-21.

86
Shumyantesva VV, Meshkov SV, Ivanov YU D, Alexandrova OV, Uvarov V YU, Archakov AI. INTERACTION OF ORGANOPHOSPHORUS ANALOGUES OF AMINO ACIDS WITH
P450. Xenobiotica 1995;25(3):219-27.

1. This study deals with the oxidation of organophosphorus amino acid analogues by phenobarbital-induced rabbit liver microsomes. It has been shown that 1-aminoalkylphosphonous and 1 -aminoalkylthiophosphonic acids are converted by P450 to 1-aminoalkylphosphonic acids. 2. Phosphonous analogues of amino acids cause type I spectral changes, and thiophosphonic analogues produce reverse type I changes in difference spectra. 3. In the presence of NADPH, the 1-aminoalkylphosphonous acids form the corresponding 1-aminoalkylphosphonic acids by the reaction P H - P OH, as monitored using 1H nmr spectroscopy. 4. Aminoalkylthiophosphonic acids have also proven to be the substrates for the NADPH-dependent monoxygenase system. During the course of oxidative desulphuration 1-aminoalkylphosphonic acids were formed by the reaction P S - P O, as monitored by 31P-nmr spectroscopy. 5. Using resonance Raman (RR) spectroscopy, the interaction of 1-aminoisobutylphosphonous acid with P450 was investigated, and characteristic changes in spectral frequencies in the region between 1370 and 1700 cm-1 were demonstrated. These latter changes indicate that substrate binding of organophosphorus compounds leads to alterations in haem conformation and to redistribution of the electron density.

87
Shackleton GL, Gibson GG, Sharma RK, Howes D, Orrenius S, Kass GE. DIVERSE MECHANISMS OF CALCIUM MOBILIZATION BY PEROXISOME PROLIFERATORS IN RAT HEPATOCYTES. Toxicol Appl Pharmacol 1995; 130(2):294-303.

The ability of six peroxisome proliferators to modulate Ca2+ homeostasis was studied in freshly isolated rat hepatocytes. Clofibrate and bifonazole (0.5 mM) caused a transient increase in cytosolic-free Ca2+ concentration ((Ca2+)i) by releasing the intracellular inositol 1,4,5-trisphosphate-sensitive Ca2+ pool. However, the mobilization of this pool by clofibrate was only transient; a subsequent exposure of the cells to the endoplasmic reticulum Ca2+-ATPase inhibitor thapsigargin resulted in a second release of the same Ca2+ store, indicating that this pool could refill from the cytosol, independently of extracellular Ca2+. By contrast, bifonazole-exposed hepatocytes no longer responded to a stimulation by thapsigargin. Bifonazole also strongly inhibited Ca2+ influx. Ciprofibrate and nafenopin (0.5 mM) produced increases in (Ca2+)i that were sustained, even in the absence of extracellular Ca2+. The (Ca2+)i response was not due to release of the inositol 1,4,5-trisphosphate-sensitive Ca2+ pool and was not inhibited by prior treatment with the protonophore carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone, but was slightly antagonized by prior exposure to the Ca2+ ionophore ionomycin. Pretreating the cells with nafenopin completely abolished the response elicited by ciprofibrate, and vice versa. By contrast to the other peroxisome proliferators, WY-14,643 and bezafibrate (1 mM) increased cytosolic free Ca2+ only by approximately 30 nM. In conclusion, the structurally diverse

peroxisome proliferators tested in this study all produced changes in (Ca2+)i in hepatocytes but through the redistribution of different internal Ca2+ pools. Further studies are needed to determine whether any of the observed Ca2+ changes have a role in the pleiotropic effects elicited by peroxisome proliferators.

88
Tanaka T, Tanigawa T, Nose T, Imai S, Hayashi Y. IN VITRO CYTOTOXICITY OF SILICIC ACID IN COMPARISON WITH THAT OF SELENIOUS ACID. J Trace Elem Exp Med 1994-1995;7(3):101-11.

Cytotoxic effects of silicic acid were investigated in two mouse cell line systems of a macrophage cell line (A640-BB-2 cells) and a fibroblast cell line (3T6 cells), in comparison with those of selenious acid. Phagocytic activity of the A640-BB-2 cells incubated for 48 h was significantly decreased at 2.0 mmol/l of silicic acid or 0.01 mmol/l of selenious acid in the culture medium, as compared to control culture with medium alone. During 48 h incubation, proliferation of the 3T6 cells was depressed at 2.0 mmol/l of silicic acid or 0.04 mmol/l of selenious acid in the culture medium. Comparison between silicon and selenium contents of cells incubated at the minimum toxic level of medium Si(OH)4 or H2SeO3 indicated the silicon content to be 500 times higher in A640-BB-2 cells and 300 times higher in 3T6 cells than the selenium content. These results indicate that the cytotoxicity of silicic acid on macrophages and fibroblasts is far weaker than that of selenious acid.

89
Petite H, Duval J, Frei V, Abdul-Malak N, Sigot-Luizard M, Herbage D. CYTOCOMPATIBILITY OF CALF PERICARDIUM TREATED BY GLUTARALDEHYDE AND BY THE
ACYL AZIDE METHODS IN AN ORGANOTYPIC CULTURE MODEL. Biomaterials 1995;16(13):1003-8.

Glutaraldehyde (GTA) is used to crosslink collagen-based biomaterials, but these materials are often cytotoxic. In order to overcome this problem, the use of the acyl azide methods with either hydrazine or diphenylphosphoryl azide (DPPA) as reagents was proposed. The cytocompatibility of acyl azide- and GTA-treated pericardium was evaluated in vitro by an organotypic chick aorta culture technique developed for the evaluation of the propensity of vascular cells (both endothelial and smooth muscle cells) to migrate and growth on the surface of biomaterials. Pericardium stabilization was examd. as a function of GTA concn. and time, so that the residual GTA mols. in the material were minimized. Treatment for 72 h with 0.95% GTA was optimal for thermal stabilization of the pericardium with a denaturation temp. (Td) of 86.8.degree., providing similar results to treatment wit 0.6% GTA for 4 h (Td -85.1.degree.). Pericardium treated in this way was, however, poorly cytocompatible with little vascular cell migrating and growth when compared with tissues treated by the acyl azide methods. The best results were obtained

with 0.5% DPPA; treated tissues showed a high level of crosslinking (Td = 82.4.degree.) and three-fold increases in cell growth and migration over those

in a non-toxic control.

90
Sakai A, Yamakoshi YN, Miyata N. THE EFFECTS OF FULLERENES ON THE INITIATION AND PROMOTION STAGES OF BALB/3T3 CELL TRANSFORMATION. Fullerene Sci Technol 1995;3(4):377-88.

Fullerene C60 and a mixt. of fullerenes C60 and C70 were solubilized in a culture medium with poly(vinylpyrrolidone) and examd. for the effects on

two-stage cell transformation in vitro, a model of carcinogenesis. They showed neither initiating nor promoting activity in the assays.

91
Attawia MA, Devin JE, Laurencin CT. IMMUNOFLUORESCENCE AND CONFOCAL LASER SCANNING MICROSCOPY STUDIES OF OSTEOBLAST GROWTH AND PHENOTYPIC EXPRESSION IN
THREE-DIMENSIONAL DEGRADABLE SYNTHETIC MATRIXES. J Biomed Mater Res 1995;29(7):843-8.

In the development of three-dimensional cell-polymer synthetic matrixes for tissue regeneration, visualization of cells growing in these porous structures can be difficult. The focus of this study was the development and use of a novel method that would allow for visualization of osteoblasts inside opaque matrixes. The morphol. responses and phenotypic characterization of osteoblasts as they attach, spread, and migrate through a porous three-dimensional biodegradable polymer-ceramic matrix in vitro were studied using immunofluorescence and confocal laser scanning microscopy (CLSM). CLSM offers several advantages over the most commonly used imaging methods (traditional light microscopy and SEM). CLSM filters out-of-focus background and provides more structural details of cells. In addn., CLSM does not require extensive sample prepn. as does SEM. When used in conjunction with fluorescence-labeled antibodies to identify cells and their products, it can characterize morphol. of growing cells and successfully det. phenotypic function. Using monoclonal antibody to osteocalcin, a bone cell-specific protein, cells throughout the matrix were found to have preserved osteoblast-like phenotype with growth. The morphol. of cells throughout the matrix was found to be similar to osteoblast cells grown on tissue culture polystyrene and consisted of spread polygonal forms. Using the technique of CLSM with immunofluorescent antibodies, it was demonstrated for the first time that these three-dimensional degradable polymer matrixes can support osteoblast growth and phenotypic expression throughout its structure.

92
Smith CG, Lee SJ, Marquardt DL. EFFECTS OF TUBERCIDIN AND ITS 5'-O-METHYL ETHER ON ADENOSINE RECEPTORS AND MEDIATOR RELEASE FUNCTIONS IN MAST CELLS. J
Med Chem 1995;38(12):2259-62.

Tubercidin (7-deazaadenosine, Tu) is a highly cytotoxic nucleoside xenobiotic that, as the nucleoside or nucleotide derivs., closely mimics the actions of

adenosine (or its corresponding nucleotides) in a wide variety of biochem./biol. systems. In light of its acceptance in these test systems as an adenosine (Ado) surrogate, it was postulated that the compd. might interact with adenosine receptors. To test this hypothesis, a nonphosphorylatable deriv. (5'-O-Me tubercidin, MeTu) was prepd. and evaluated in comparison with tubercidin and Ado in a variety of biol. systems. In a cell culture assay using Chinese hamster ovary cells, MeTu is approx. one-third as cytotoxic as is Ado and 105-fold less cytotoxic than Tu. Both Tu and MeTu inhibited the antigen-stimulated release of beta-hexosaminidase from mouse bone marrow derived mast cells in vitro, but only Tu was active in the in vivo PCA test. The inhibitory effect of MeTu on mast cell mediator release does not appear to involve interaction with adenosine receptors or to be the result of conversion to Tu per se.

93
Zhang BT. [EFFECTS OF MITOGENS ON ADULT SCHWANN CELLS ISOLATED FROM MOUSE
SCIATIC NERVE.] Yokohama Igaku 1995;46(2):111-7. (Jpn)

We developed a new method of extg. adult Schawann cell (SCs) from the mouse sciatic nerve and investigated the effects of mitogens on adult SCs in vitro. This culture system provided 99.5% pure SCs populations at cell yields of greater than 3 .times. 103 cell/mg of starting nerve wet wt. at 5 culture days by the following method (1) culture the dissocd. adult SCs for 24 h in 10% FCS-F12 medium, (2) culture these cells in serum-free medium for 48 h, (3) differential adhesion step. We examd. proliferating response of adult SCs to 3 identified neonatal SCs mitogens: glial growth factor (GGF), platelet-derived growth factor BB (PDGF-BB), and basic fibroblastic growth factor (bFGF) in serum-free medium. GGF alone had mitogenicity for adult SCs in a dose-dependent manner, and synergistic activation coupling with forskolin was not obsd. Neither PDGF-BB nor bFGF was mitogenic for adult SCS when used alone or with forskolin. GGF and bFGF did not change the cAMP levels in SCs. However, PDGF-BB induced one half lower level than basal cAMP level. These results indicate that cAMP was not related with DNA synthesis of SCs in GGF

response, and GGF activate adult SCs through a signal transduction pathway sep. from the cAMP-dependent system.

94
Rigano L, Cavalletti T, Benetti S, Traniello S. EVALUATION OF PREDICTABLE IRRITATIVE POWER OF SURFACTANT MIXTURES BY HUMAN FIBROBLAST CULTURE AND ITS
CORRELATION TO PHYSICO-CHEMICAL PARAMETERS. Int J Cosmet Sci 1995;17(1):27-43.

The predictable toxic effects of some surfactants, their blends and some preserving agents on human fibroblast cultures were investigated with in vitro tests, with the aim of finding a possible correlation between the biol. evaluations and some phys. characteristics of detergent solns. Lactate dehydrogenase release into the medium was used as a marker of the plasma membrane integrity, while the amt. of 3H-radiolabeled proteins in the fibroblasts was measured in order to assess the cell biosynthetic machinery

function. Disodium alkyl semisulfosuccinate induced membrane damage in the lactate dehydrogenase test and decreased the protein synthesis, with an EC 50 around 1 mM, while sodium lauryl ether sulfate had an EC 50 at about 100 muM, indicating that this compd. is ten-fold more toxic, when measured by this method. An ethoxylated glyceride, on the contrary, was completely harmless on the plasma membrane and, surprisingly, activated fibroblast protein synthesis in a dose-response way up to two-fold. Mixts. of the three surfactants evidenced the protective effect of the non-ionic against the cellular functionality damage. Parabens do not influence this type of evaluation, while some influence was shown by the formaldehyde releaser 2-bromo-2-nitropropanediol at the highest concn. The comparison between crit. micellar concn. measures of the different surfactants and their in vitro detected irritative power shows, for the two anionics, that in vitro toxicity is proportionally bound to the amt. of micelles even if the structural differences between the two types of mols. are reflected into different damage values, while the non-ionic compd. shows a not defined CMC and a very low toxicity profile. Blends of anionics with the non-ionic show an increased CMC and a reduced toxicity profile. Toxicity evaluations of complex finished foaming formulations, carried out with human fibroblast cultures evaluation show that a relationship between micelles amt. and cell toxicity seems to exist, mainly when multiple surfactants blends are tested.

95
Carlson SL, McGillis JP. MODULATION OF LEUKOCYTE ADHESION, MIGRATION, AND HOMING BY NEOROTRANSMITTERS AND NEUROPEPTIDES. Methods Neurosci 1995;24:335-54.

A review describing cell culture methods for endothelial cells (from human umbilical vein, mouse lymphoid microvessels, and rat heart microvessels) and in vitro adhesion assays using these endothelial cells, in vivo assays of leukocyte homing to lymphoid tissues, and tissue-specific lymphocyte migration assays. These methods are used to det. the effects of catecholamines, neuropeptide Y, substance P, calcitonin gene-related peptide, and other neurotransmitters found in nerve terminals in lymphoid tissue and surrounding blood vessels.

96
Lindl T. CELL SYSTEMS ON THE ADVANCE. PART 1. CLB. Chem Labor Biotech 1994;45(5):258-61.

CYTOTOXICITY
97
Liebsch HM, Spielmann H. BALB-C 3T3 CYTOTOXICITY TEST. Methods Mol Biol 1995;43:177-87.

98
Guigand M, Pellen P, Mouton C, Bonnaure-Mallet M. CYTOTOXIC EFFECT OF VESICLES PRODUCED BY PORPHYROMONAS GINGIVALIS ON FIBROBLASTS IN CULTURE. J
Periodont Res 1995;30(2):141-3.

It has been shown that the vesicles produced by Porphyromonas gingivalis under certain growth conditions contribute to its pathogenicity. In this study, we demonstrate the cytotoxic effect of the vesicles using two methods: one quantitative (the colorimetric cytotoxicity test using sulforhodamine B) and the other qualitative (flow cytometry).

99
Brambilla G, Marteili A. CYTOTOXICITY DNA FRAGMENTATION AND DNA REPAIR SYNTHESIS IN PRIMARY HUMAN HEPATOCYTES. Methods Mol Biol 1995;43:59-66.

100
Zeromski J, Jezewska E. FUNCTIONAL ALTERATIONS OF HUMAN BLOOD MONOCYTES AFTER
EXPOSURE TO VARIOUS NICKEL COMPOUNDS IN VITRO: AN EFFECT ON THE PRODUCTION OF
HYDROGEN PEROXIDE. Immunol Lett 1995;45(1-2):117-21.

It is generally known that nickel, a metal with distinct carcinogenic properties, can significantly alter the functioning of host defense mechanisms and impair various components of the immune system. In the present study the influence of 3 nickel salts on the production of hydrogen peroxide (H2O2) by human monocytes was examined in in vitro culture. Highly purified, resting and PMA-stimulated normal human monocytes were cultured with subtoxic concentrations of nickel subsulfide, nickel sulfate, nickel acetate and manganese chloride. A portion of the cells was cultured with nickel-manganese salt mixture. Following culture cells were tested in an in vitro functional assay for H2O2 production. It has been shown that all nickel salts, used in micromole concentrations, suppressed H2O2 formation both in resting and PMA-stimulated monocytes, while it was not the case when manganese chloride was used for cell cultures. The strongest suppressive effect was manifested by nickel sulfate. The cells subjected to nickel-manganese mixture displayed H2O2 production similar to that of control ones. These results show that nickel salts in micromole concentrations exert a suppressive effect on oxygen-dependent antimicrobial system of human monocytes and manganese prevents this effect.

101
Nichols WK, Terry CM, Cutler NS, Appleton ML, Jesthi PK, Yost GS. OXIDATION AT C-1 CONTROL THE CYTOTOXICITY OF 1,1-DICHLORO-2,2-BIS(P-CHLOROPHYNYL)ETHANE
BY RABBIT AND HUMAN LUNG CELLS. Drug Metab Dispos 1995;23(5):595-9.

Isolated rabbit Clara cells and a transformed human bronchial epithelial cell line, BEAS-2B, were used to investigate the mechanism of cytotoxicity of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane DDD), a persistent insecticide and stable metabolite of 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane. Both BEAS-2B cells and rabbit Clara cells were highly susceptible to DDD toxicity and were partially protected by 1-aminobenzotriazole, a suicide substrate inhibitor of cytochrome P450 enzymes. DDD (0.05 mM) killed 47 : 1.8% of rabbit Clara cells and 42 : 7.9% of BEAS-2B cells after 3 hr and 84 : 3.0% of

rabbit Clara cells and 80 : 14% of BEAS-2B cells after 6 hr. Consequently, DDD is the most potent Clara cell toxicant recognized to date. The cytotoxicity of DDD to these cells was decreased by deuterium substitution at the C-1 position. Rabbit Clara cells and pulmonary microsomes incubated with 14C-DDD produced the fully oxidized acetic acid metabolite 2,2'-bis(p-chlorophenyl)acetic acid (DDA), but DDA was not formed by Clara cells when DDD was coincubated with 1-aminobenzotriazole. These results support the hypothesis that the cytotoxicity of DDD to susceptible subpopulations of rabbit and human lung cells is, at least in part, caused by cytochrome P450-mediated oxidation of DDD at C-1. A required step for the production of the cytotoxic intermediate is proposed to be the formation of a highly reactive acyl halide intermediate that is readily hydrolyzed to a stable, nontoxic metabolite, DDA.

102
Tu B, Wallin A, Moldeus P, Cotgreaveia. CYTOTOXICITY OF NO2 GAS TO CULTURED HUMAN AND MURINE CELLS IN AN INVERTED MONOLAYER EXPOSURE SYSTEM. Toxicology
1995;96(1):7-18.

We report the development of an optimised exposure system for the exposure of inverted cell cultures to NO2, which presents several advantages over conventional, right-side-up exposure systems. Firstly, the cells may be directly exposed to NO2 in the gas phase for up to 1 h, without the interposition of an aqueous layer. Secondly, the chamber system allows simple and precise control of the gas concentration during the exposure. Finally, the system allows the simultaneous exposure of large numbers of cells under sterile conditions, facilitating further culture of the cells after the exposure period. We report the application of this system to a comparative study of the toxicity of NO2 in three different cell types involved in the circuit of the inflammatory response, the IC-21 murine macrophage line, the A-549 human pulmonary type II-like epithelial cell line and human umbilical vein endothelial cells. As little as 2 ppm NO2 for 20 min reduced colony-forming efficiency of HUVE cells and A-549 cells to 35% and 78% of their air controls, respectively. Exposure to 5 ppm NO2 for 1 h increased lactate dehydrogenase release of HUVE cells, IC-21 macrophages and A-549 cells from 7.9% to 21.6%, 5.7% to 10.9% and 2.0% to 3.4%, respectively, whilst 10 ppm NO2 for 1 h lowered cellular glutathione in HUVE cells, IC-21 cells and A-549 cells from 35.2 nmol/mg to 23.3 nmol/mg, from 45.0 nmol/mg to 31.0 nmol/mg and from 86.4 nmol/mg to 69.2 nmol/mg, respectively. Of the cell types

tested it was shown that HUVE cells and IC-21 cells were equally sensitive to the toxicity of NO2, whilst A-549 cells displayed considerable resistance, perhaps due to the considerably higher levels of glutathione in this cell line. Further, a comparison of the sensitivity of HUVE cells to NO2, using several modes of exposure (inverted and right-side-up (either rocked or static)) and the assay of lactate dehydrogenase and (3H)deoxyglucose release, revealed that the present inverted exposure technique potentiated the acute cytotoxicity of the gas.

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Rath NC, Huff WE, Bayyari GR, Balog JM. EFFECT OF THIRAM ON CHICK CHONDROCYTES IN CULTURE. J Toxicol Environ Health 1995;44(3):369-76.

The effect of thiram, a fungicide that increases the incidence of tibial dyschondroplasia (TD) in poultry, was studied in vitro using growth plate chondrocyte culture. Thiram caused a significant reduction in alkaline phosphatase, acid phosphatase, and lactate dehydrogenase (LDH) activities at concentrations of 5 muM and above. It was highly cytotoxic to chondrocytes at and above this concentration as determined by their ability to reduce 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (triazolyl blue, MTT), a marker of cellular viability. An increase in the leakage of LDH into culture media was evident at concentration as low as 1 muM. Very few differences were noticed in the electrophoretic migration profiles of cell-extract proteins at any treatment level relative to control. The cytotoxic effect of thiram is possibly due to its damaging effect on the cell membrane, which may be responsible for chondrocyte death.

104
Dunn K LR, Virji M, Moxon ER. INVESTIGATIONS INTO THE MOLECULAR BASIS OF MENINGOCOCCAL TOXICITY FOR HUMAN ENTOTHELIAL AND EPITHELIAL CELLS: THE SYNERGISTIC EFFECT OF LPS AND PILI. Microb Pathog 1995;18(2):81-96.

Using human umbilical vein endothelial cells as an in vitro model of toxicity, it was found that Neisseria meningitidis, Neisseria gonorrhoeae, Neisseria lactamica and Neisseria sicca caused damage to these cells, in contrast to the lack of cytotoxicity exhibited by Haemophilus influenzae type b. N. meningitidis was also found to be toxic for human epithelial cells. The major toxic factor of N. meningitidis was found to be a heat-stable component of outer membrane vesicles, and could be inhibited by polymyxin B, suggesting that lipopolysaccharide plays a major role in toxicity. However, the toxicity mediated by lipopolysaccharide was modulated significantly by pilus-dependent adherence. Intra-strain variants expressing altered pilins which exhibited different levels of adherence to epithelial and endothelial cells were used to study the role of pilus. The degree of toxicity observed correlated with their relative level of adherence to cultured cells. In contrast, Opc-dependent increased adherence did not result in increased toxicity for endothelial cells, suggesting that pili have a synergistic effect, contributing to the overall damage.

105
de Arruda M, Cocchiaro CA, Nelson CM, Grinnell CM, Janssen B, Haupt A, Barlozzari T. LU103793 (NSC D-669356): A SYNTHETIC PEPTIDE THAT INTERACTS WITH MICROTUBULES AND INHIBITS MITOSIS. Cancer Res 1995;55(14):3085-92.

LU103793 (NSC D-669356) is a new synthetic derivative of Dolastatin 15, an antiproliferative compound which was isolated from the mollusk Dolabella auricularia. Like Dolastatin 15, LU103793 is highly cytotoxic in vitro (IC50 = 0.1 nM). To investigate the mechanism of action of LU103793, we used a combination of biochemical and cellular methods. Turbidity assays with bovine brain microtubules demonstrated that LU103793 inhibits microtubule polymerization in a concentration-dependent manner (IC50 = 7 microM).

Treatment with this compound also induced depolymerization of preassembled microtubules. Cell cycle analysis of tumor cell lines treated with LU103793 indicated a block in the G2-M phase. At the cellular level, it induced depolymerization of microtubules in interphase cells and development of abnormal spindles and chromosome distribution in mitotic cells. Although these effects are very similar to the cellular alterations caused by vinblastine, LU103793 does not inhibit vinblastine binding to unpolymerized tubulin in vitro. Our results suggest that LU103793 exerts its cytotoxic activity primarily through disruption of microtubule organization.

106
Fridborg H, Nygren P, Larsson R. RELATIONSHIP BETWEEN PHARMACOKINETIC PARAMETERS IN PATIENTS AND CYTOTOXICITY IN VITRO OF STANDARD AND INVESTIGATIONAL ANITCANCER DRUGS. Anticancer Drugs 1995;6(1):64-9.

The selection of the starting dose for initial clinical trials of anticancer agents is mostly determined by toxicological endpoints in mice (LD10). So far, very few attempts have been made to evaluate the potential value of cytotoxicity assays for this purpose. The present study was undertaken as a first attempt to investigate the relationship between cytotoxicity of anticancer drugs in vitro and pharmacokinetic parameters in vivo in patients, at suggested maximum tolerated doses. Using the fluorometric microculture cytotoxicity assay (FMCA), we determined the concentration giving 50% cell survival (IC50) in vitro, for 25 cytotoxic drugs in fresh preparations of normal peripheral blood mononuclear cells (PBMC) and of tumor cells from patients with acute or chronic lymphocytic leukemia (ALL or CLL). Using linear regression, we investigated the relationship between the IC50s and clinically achievable peak plasma concentrations (Cmax) or concentration-time products (C x T) in humans. The clinical data was obtained from the literature. Based on all drugs tested, good correlations were obtained between IC50s for CLL cells, and both Cmax and C x T (R approximately 0.7, $p < 0.0002$), and for ALL cells and normal PBMC between IC50 and Cmax, while the two latter cell types showed somewhat weaker relationships to C x T. Using the IC50 data of CLL cells, predictions of Cmax and C x T exceeded 1 log for only four drugs. No tendencies to under- or overprediction within different classes of drugs were noted. The results demonstrate a significant relationship between toxicity in vitro and achievable systemic exposure of anticancer drugs in humans, which suggests that non-clonogenic in vitro assays for drug sensitivity testing may provide pharmacokinetic information useful in the development of investigational cytotoxic drugs.

107
Fischel JL, Barbe V, Berlion M, Formento P, Berrile J, Bizzari JP, Milano G. [TAMOXIFEN INCREASES CYTOTOXIC EFFECTS OF FOTEMUSTINE. EXPERIMENTAL RESULTS ON
CELL LINES OF HUMAN MELANOMA.] Bull Cancer 1994; 81(7):599-604. (Fre)

Fotemustine (Fote) is a new aminoacid linked chloroethyl nitrosourea which has been shown to be useful in disseminated malignant melanoma. The aim of the present study was to analyze the cytotoxic effects resulting from the

combination of antiestrogens and Fote on human melanoma cell lines. The antiestrogens tested were tamoxifen (TMX 5.10(-7) M and 5.10(-6) M) and 40H TMX (5.10(-8) M and 5.10(-7) M). As a preliminary step, a series of nine human melanoma cell lines was screened in order to identify and quantify the presence of estradiol receptors (ER) in these cell lines. This led to selecting an ER positive (+) cell line. The drugs alone or in combination were then tested against CAL 1 ER (+) and CAL 7 ER (-) melanoma cell lines. Different sequences of drug combinations were tested using clinically compatible drug concentrations. For CAL 1 cells there was a growth inhibitory effect induced by the antiestrogens given alone. Overall, the presence of the antiestrogens resulted in higher cytotoxic effects than when cells were exposed to Fote alone. The lowest IC50 Fote values as compared to Fote alone were generated by the sequences with the antiestrogens administered before Fote. Significantly, these associations with antiestrogens enabled the IC50 values of Fote to be reduced up to 80%. Globally TMX and 40H TMX had similar synergistic effects. TMX and 40H TMX had a modest influence on Fote cytotoxic effects against CAL 7 ER (-) cells. These data may be useful for optimal planning of future clinical trials for malignant melanoma using antiestrogens and nitrosoureas.

108
Copaceanu ML, Coucke PA, Cottin E, Paschoud N, Mirimanoff RO. AZIDOTHYMIDINE (AZT) AS A POTENTIAL MODIFIER OF RADIATION RESPONSE IN VITRO. Acta Oncol 1995;34(2):213-8.

The potential effect of AZT as a thymidine analogue on radiation response in vitro was investigated. Two human cell lines (WiDr and HeLa) were used. The effect of 10 microM AZT on exponentially growing cells was studied after different exposure times (24, 48 and 72 h). The surviving fraction (clonogenic assay) or metabolic activity (MTT assay) after irradiation of AZT-exposed cells, was compared to unexposed irradiated controls. Flow cytometry was used to assess the cell-cycle effect of pre-exposure of exponentially growing cells to AZT. AZT had a radioprotective effect for all experimental time points as far as WiDr was concerned. For HeLa the effect was significant at 24 h. Cell-cycle analysis showed a significant accumulation in S-phase at 72 h for WiDr. For HeLa there was a significant accumulation in S-phase at 48 h. We conclude that under the reported experimental conditions, AZT as a thymidine analogue seems to reduce the cytotoxic effect of irradiation.

109
Yourtee DM, Tong PY, Rose LA, Eick JD, Chappelow CC, Bean TA. THE EFFECT OF SPIROORTHOCARBONATE VOLUME MODIFIER CO-MONOMERS ON THE IN VITRO TOXICOLOGY OF
TRIAL NON-SHRINKING DENTAL EPOXY CO-POLYMERS. Res Commun Mol Pathol Pharmacol 1994;86(3):347-60.

A major improvement in dental restoratives is possible through the development of biomaterials that do not shrink upon polymerization, hence, avoid leakage and subsequent breakdown. Polymers containing spiroorthocarbonates (SOCs) show promise in this respect, but their toxicology in copolymerized materials has not been explored. In this study, the in vitro toxicology of these materials

in homopolymer form and in two trial non-shrinking epoxy co-polymers was evaluated for cytotoxicity and mutagenicity. Cytotoxicity was determined by the MTT test to measure the lethality effect on mouse L929 cells. Mutagenicity was evaluated using the Ames-Salmonella Test. For comparison, commercial composite and adhesive materials as well as several other materials of current interest in dentistry were also evaluated. Epoxy resin samples containing 5% of either T/T SOC or Dp SOC reduced the cytotoxicity (TC50) from approximately 400 to 800 micrograms/200 microliters. The epoxy-spiro copolymers had more favorable TC50 values than the commercial product Super-Bond. They showed TC50 values on the order of 35% greater than Super-Bond and 45% less than Scotchbond 2, the latter two being materials currently used in the clinic. These two comparatives demonstrated dose response curves with lower doses at maximum cell kill values than the spiro materials. The epoxy formulations all showed weak mutagenesis, but this is attributed to the epoxy formulation and not the SOCs. Although considerable toxicology is yet be conducted, these in vitro results suggest that biocompatible copolymer formulations for spiroorthocarbonates are a developmental reality.

110
Fos E, Suesa N, Borras L, Lobato C, Banfi P, Gambetta RA, Zunino F, Mauleon D, Carganico G. SNYTHESIS OF ALKYL CHAIN-MODIFIED ETHER LIPIDS AND EVALUATION OF THEIR IN VITRO CYTOTOXICITY. J Med Chem 1995;38(7): 1216-28.

A series of alkyl lysophospholipid (ALP) analogs of ET-18-OCH3 (1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine) containing modifications in the long C-1 chain has been synthesized and evaluated in human tumor cell line cytotoxicity assays. The compounds have also been evaluated in platelet activating factor (PAF) receptor agonism and hemolysis tests. Two modifications have been studied, introduction of a carbonyl group at different positions of the C-1 chain and branching of this chain, in some compounds with incorporation of a phenyl group. Several compounds showed a cytotoxic potency comparable to that of the reference compound ET-18-OCH3, associated with reduced proaggregating and hemolytic effects. The two enantiomers of 1-O-(7-oxooctadecyl)-2-O-methyl-rac-glycero-3-phosphocholine (2) showed the same level of cytotoxicity or antiproliferative activity, with the PAF-agonistic effect confined to R-2. The very low stereoselectivity found in the in vitro cytotoxicity confirms earlier results and indicates a lack of stereospecific interactions with a macromolecular target.

111
DeCesare SL, Michelini-Norris B, Blanchard DK, Barton DP, Cavanagh D, Roberts WS, Fiorica JV, Hoffman MS, Djeu JY. INTERLEUKIN-12-MEDIATED TUMORICIDAL ACTIVITY OF PATIENT LYMPHOCYTES IN AN AUTOLOGOUS IN VITRO OVARIAN CANCER ASSAY
SYSTEM. Gynecol Oncol 1995;57(1):86-95.

This study was designed to examine if interleukin-12 (IL-12) can induce cytolytic function of lymphocytes from ovarian cancer patients against either an ovarian cancer cell line or their own autologous tumor cells. Lymphocytes were obtained from the peripheral blood or ascites of ovarian cancer patients and activated with IL-12 alone or concomitantly with interleukin 2 (IL-2) for

2 to 3 days. Activation of lymphocytes and assessment of tumoricidal function by a chromium release assay were performed directly in a standard control medium (RPMI 1640 containing 2 mM glutamine, 100 micrograms/ml streptomycin, 100 units penicillin, 5% heat-inactivated human AB serum, and 5 mM 4-(2-hydroxyethyl)-1-piperazinesulfonic acid) and in 50% ascitic fluid (50% by volume filter-sterilized ascites with 50% of the above-mentioned control medium). Target cells were added directly into the medium in which the lymphocytes were activated in order to more closely mimic in vivo conditions. Lymphocytes, activated by IL-12 in 50% ascitic fluid, were able to lyse autologous tumor cells in 3 of 6 assays and were able to lyse SKOV3 cells (an ovarian cancer cell line) in 5 of 7 assays. The results were not significantly different in the control medium. When both IL-2 and IL-12 were used to activate lymphocytes in 50% ascitic fluid, significant cytotoxicity was generated in 6 of 6 autologous assays and in all 7 patient assays using SKOV3 as a target ($P < 0.05$). Synergy between the two cytokines was seen in all 13 patient assays in ascitic medium compared to only 5 of 13 assays in control medium. Additionally, when lymphocytes were stimulated with both IL-2 and IL-12, significantly greater cytotoxicity was seen in the ascitic fluid medium compared to the control medium in 13 of 14 assays ($P < 0.05$). No significant tumoricidal activity was seen by lymphocytes maintained in either medium without the addition of IL-2 or IL-12. Ascitic fluid consistently potentiates the synergy between IL-2 and IL-12 in generating cytotoxicity against ovarian cancer cells but does not increase cytotoxicity induced by IL-12 alone. IL-12 by itself activates tumoricidal activity of lymphocytes in ascitic fluid; however, the addition of IL-2 increases the degree and consistency of this effect. These data support the possibility that IL-12 may warrant further investigation as a potential therapeutic agent in the treatment of advanced ovarian cancer.

112
Kaspers GJ, Veerman AJ, Pieters R, Van Zantwijk I, Hahlen K, Van Wering ER. DRUG COMBINATION TESTING IN ACUTE LYMPHOBLASTIC LEUKEMIA USING THE MTT ASSAY. Leuk Res 1995;19(3):175-81.

Drug resistance assays may be useful to identify drug interactions. For this purpose, we studied three drug combinations, each at 8-12 concentrations, with the MTT assay in acute lymphoblastic leukemia (ALL) samples from 34 children btained at initial diagnosis. This resulted in a total of 518 comparisons between expected and observed leukemic cell survivals. The combinations prednisolone (PRD) with vincristine (VCR), PRD with mafosfamide (MAF), and PRD with daunorubicin (DNR) were tested without technical difficulties, and without an increased assay variation as compared to single drugs. We observed a marked heterogeneity in drug interactions between patients, between combinations, and between different concentrations within one specific combination. Between PRD+VCR, synergism was found in 46%, antagonism in 18%, and additivity in 36% of the 228 observations. Between PRD+MAF, synergism was found in 51%, antagonism in 20%, and additivity in 29% of the 140 observations. Between PRD+DNR, synergism was found in 35%, antagonism in 31%, and additivity in 34% of the 150 observations. PRD+VCR and PRD+MAF showed more often synergism than PRD+DNR, while antagonism was observed more frequently between PRD+DNR ($p < 0.05$). However, the magnitude of antagonism

was not much different between the three drug combinations, nor was there a significant antagonistic interaction in any of the drug combinations tested, if all samples were considered together. We conclude that the MTT assay can be used to study drug interactions in vitro in ALL samples. The type of interaction was different between patients, and depends on the drug combination and concentrations. The combinations PRD+VCR and PRD+MAF generally showed additive and even synergistic interactions. The cytotoxicity of PRD+DNR was generally not markedly higher than that of the most active single drug.

113
Timbrell JA, Seabra V, Waterfield CJ. THE IN VIVO AND IN VITRO PROTECTIVE PROPERTIES OF TAURINE. Gen Pharmacol 1995;26(3):453-62.

1. Taurine is a ubiquitous, free amino acid found in mammalian systems. 2. The biological functions of taurine are unclear. 3. Various in vivo data suggest that taurine has a variety of protective functions and deficiency leads to pathological changes. 4. Depletion in rats of taurine increases susceptibility to liver damage from carbon tetrachloride. 5. Susceptibility to a variety of hepatotoxicants correlates with the estimated hepatic taurine level. 6. In vitro data suggest that taurine can protect cells against toxic damage. 7. Taurine protects isolated hepatocytes against carbon tetrachloride, hydrazine and 1,4-naphtho- quinone but not against allyl alcohol, alpha-naphthylisothiocyanate (ANIT) or diaminodiphenyl methane (DAPM) cytotoxicity. 8. The mechanisms of protection are unclear but may include modulation of calcium levels, osmoregulation and membrane stabilization.

114
Sinensky MC, Leiser AL, Babich H. OXIDATIVE STRESS ASPECTS OF THE CYTOTOXICITY OF CARBAMIDE PEROXIDE: IN VITRO STUDIES. Toxicol Lett 1995;75(1-3):101-9.

Carbamide peroxide is the active ingredient in many at-home patient-applied tooth whiteners. The cytotoxicity of carbamide peroxide, as related to oxidative stress, was evaluated in vitro with several human cell lines, including Smulow-Glickman (S-G) gingival epithelial cells. The potency of carbamide peroxide was related to its hydrogen peroxide component rather than to carbamide, was eliminated in the presence of exogenous catalase, and was enhanced in the presence of aminotriazole, an inhibitor of cellular catalase. The intracellular level of glutathione, a scavenger of toxic oxygen metabolites, was decreased in cells exposed to carbamide peroxide; at higher concentrations of carbamide peroxide, leakage of lactic acid dehydrogenase was also evident. Cells pretreated with the glutathione-depleting agents, buthionine sulfoximine, chlorodinitrobenzene, and bis(chloroethyl) nitrosourea, were hypersensitive to subsequent challenge with carbamide peroxide. Conversely, pretreatment with the iron chelator, deferoxamine, protected the cells against subsequent exposure to carbamide peroxide.

115
Wieslander AP, Deppisch R, Svensson E, Forsback G, Speidel R, Rippe B. IN VITRO BIOCOMPATIBILITY OF A HEAT-STERILIZED, LOW-TOXIC, AND LESS ACIDIC FLUID FOR PERITONEAL DIALYSIS. Perit Dial Int 1995;15(2):158-64.

The aim of this study was to investigate a peritoneal dialysis (PD) fluid (PD-Bio), produced with the intention of reducing the amount of glucose degradation products and to increase the final pH. The heat sterilization of the fluid was performed with the glucose separated from the electrolytes. After sterilization the two solutions were combined. METHODS: The in vitro biocompatibility of PD-Bio was measured as the inhibition of cell growth of a cultured fibroblast cell line and as the stimulated release of interleukin-1 beta from cultured human mononuclear cells. The glucose degradation products were measured as UV absorbance at 228 nm or 284 nm and the concentration of aldehydes was estimated with high-performance liquid chromatography and gas chromatography. RESULTS: Our results demonstrate that in comparison to conventional PD fluids the pH of PD-Bio was increased, to about 6.5. Due to less contaminating glucose degradation products in PD-Bio, basal cytotoxicity was significantly decreased for both 1.5% and 4% glucose-containing fluids, and the stimulated release of interleukin-1 beta was normalized compared to sterile filtered controls with the same pH. UV absorbance measured at 228 nm was decreased, whereas the absorbance at 284 nm was equal to that of a conventional fluid. In PD-Bio the concentrations of formaldehyde, acetaldehyde, methylglyoxal, and 2-furaldehyde were found to be below the detection limit, whereas glyoxal was present in the same and 5-hydroxymethylfurfural (5-HMF) in higher concentrations than in conventionally produced PD fluid. CONCLUSIONS: The results demonstrate that it is possible to improve biocompatibility of PD fluids by simply changing the way the fluid is produced.

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Kumar G, Ray S, Walle T, Huang Y, Willingham M, Self S, Bhalla K. COMPARATIVE IN VITRO CYTOTOXIC EFFECTS OF TAXOL AND ITS MAJOR HUMAN METABOLITE 6 ALPHA-HYDROXYTAXOL. Cancer Chemother Pharmacol 1995; 36(2):129-35.

Taxol is metabolized by the liver microsomal cytochrome P450 enzyme system into its principal metabolite 6 alpha-hydroxytaxol (6HT). In the present in vitro studies 6HT was compared to taxol with respect to its effects on tubulin depolymerization, mitotic arrest, clonogenic survival and apoptosis in HL-60 cells. 6HT was generated by incubating taxol with human liver microsomes in a NADPH-generating system. HL-60 cells were incubated for 24 h with either taxol or 6HT, washed and placed in drug-free suspension or cultured for colony growth in agarose. For the suspension and colony culture growth of the cells, the IC50 concentrations of 6HT were 500 +/- 46 and 350 +/- 37 nM, while those of taxol were 3.2 +/- 0.2 and 2.8 +/- 0.5 nM, respectively. Immediately after a 24-h exposure of HL-60 cells to 50 nM taxol, electrophoresis of genomic DNA from HL-60 cells revealed an internucleosomal DNA fragmentation 'ladder'. In addition, 39% of the cells were arrested in mitosis and 16% showed the morphologic features of apoptosis. In contrast, an identical treatment with 6HT resulted in the mitotic arrest of only 2.8% of the cells, with 4.0% displaying apoptosis ($P < 0.01$); internucleosomal DNA fragmentation was not observed. 6HT was also significantly less effective than taxol in inhibiting the temperature-induced depolymerization of microtubules in a cell-free system. However, at equipotent concentrations, the effect of 6HT on tubulin depolymerization, mitotic arrest or apoptosis was similar to that of taxol. In addition, at concentrations of taxol or 6HT at or below their IC50, there was

little tubulin depolymerization, mitotic arrest or apoptosis. The results presented here show that the biotransformation of taxol to 6HT substantially detoxifies taxol.

117
Leteurtre F, Sackett DL, Madalengoitia J, Kohlhagen G, MacDonald T, Hamel E, Paull KD, Pommier Y. AZATOXIN DERIVATIVES WITH POTENT AND SELECTIVE ACTION ON TOPOISOMERASE II. Biochem Pharmacol 1995;49(9):1283-90.

Azatoxin was rationally designed as a DNA topoisomerase II (top2) inhibitor [Leteurtre et al., Cancer Res 52: 4478-4483, 1992] and was also found to inhibit tubulin polymerization. Its cytotoxicity is due to action on tubulin at lower concentrations and on top2 at higher concentrations. At intermediate concentrations, the combination of the two mechanisms appears antagonistic [Solary et al., Biochem Pharmacol 45: 2449-2456, 1993]. The aim of this study was to design azatoxin derivatives that would act only on tubulin or on top2. Selective targeting of top2 or tubulin was tested using top2-mediated DNA cleavage assays, and tubulin polymerization and tubulin proteolysis assays, as well as COMPARE analyses of cytotoxicity assays in the National Cancer Institute in vitro Drug Screening Program. Selective inhibitors of top2 and tubulin polymerization have been obtained. Top2 inhibition, abolished by methylation at position 4', was enhanced by the addition of a bulky group at position 11. Bulky substitution at position 11 determined different patterns of top2 cleavage sites and suppressed the action on tubulin. Selective inhibition of tubulin was obtained with 4'-methylazatoxin that was found to bind to the colchicine site. These results are consistent with those obtained in the podophyllotoxin family to which azatoxin is structurally related. Some azatoxin derivatives are under consideration for further preclinical development.

118
Schmalz G, Schweikl H. CHARACTERIZATION OF AN IN VITRO DENTIN BARRIER TEST USING A STANDARD TOXICANT. J Endod 1994;20(12):592-4.

To characterize a new artificial pulp chamber using bovine dentin, the influence of different phenol concentrations on the cell reaction was evaluated. Bovine dentin slices of 100, 200, 300, 500, and 700 microns were cleaned, sterilized, and mounted between two 5-mm-thick glass plates with a borehole of 5 mm in diameter. L-929 mouse fibroblasts were seeded into the "pulpal" part of the device and incubated for 24 h. Different concentrations of a phenol solution as a standard toxicant were applied to a sterile cotton pellet in the "cavity" side of the device. After incubation for 24 h, the cytotoxic reaction was evaluated by cell counting after vital staining with fluorescein diacetate and related to the negative control. A dependency of the cytotoxic reaction on the concentration of the standard toxicant and on the thickness of the dentin slice was demonstrated. The simple dentin barrier test using bovine dentin may be a suitable alternative to other more complicated procedures using human dentin.

119
Wataha JC, Hanks CT, Sun Z. EFFECT OF CELL LINE ON IN VITRO METAL ION

CYTOTOXICITY. Dent Mater 1994;10(3):156-61.

The choice of cell line for in vitro biological tests which assess the cytotoxicity of dental materials remains controversial, yet this issue is important because these tests are widely used to rate the biocompatibility of new and existing materials, and many different cell lines are commonly used. The purpose of the current study was to quantify the responses of four cell lines (Balb/c 3T3, L929, ROS 17/2.8 and WI-38) to 14 metal ions which are released from dental materials, and relate these responses to the metabolic activity and population doubling times of these cells. METHODS. Succinic dehydrogenase (SDH) activity was used to monitor metabolic activity and cytotoxic response. RESULTS. The cell lines responded differently to most metal ions. In general, the Balb/c 3T3 line was the most sensitive, and the WI-38 line was the least sensitive. However, there were many exceptions depending on the metal ion. The passage number of the cells also affected the cytotoxic response. It was concluded that the cytotoxicity of materials which release metal ions will be significantly different depending on which cell line is selected and its passage number. SIGNIFICANCE. Based on the findings that cell lines ranked the toxicities of the metal ions similarly, it seems reasonable to use these types of in vitro tests to rank the cytotoxicities of materials. However, if these types of tests are used to predict in vivo cytotoxicity, care should be taken to choose conditions and cells which are relevant.

120
Zoli W, Flamigni A, Frassineti GL, Bajorko P, De Paola F, Milandri C, Amadori D, Gasperi-Campani A. IN VITRO ACTIVITY OF TAXOL AND TAXOTERE IN COMPARISON WITH DOXORUBICIN AND CISPLATIN ON PRIMARY CELL CULTURES OF HUMAN BREAST CANCERS. Breast Cancer Res Treat 1995; 34(1):63-9.

The in vitro activities of taxol and taxotere in comparison with cisplatin and doxorubicin were assessed in 30 primary tumor cultures from human breast cancers. Both taxanes were much more potent than cisplatin and doxorubicin. Taxotere was 3.1; 296, and 9.6-fold more cytotoxic than taxol, cisplatin, and doxorubicin respectively. The cytotoxic activity observed in our experiments confirms the potential clinical relevance of the two taxanes in the management of breast cancer.

121
Sami SM, Dorr RT, Solyom AM, Alberts DS, Remers WA. AMINO-SUBSTITUTED 2-[2'-(DIMETHYLAMINO)ETHYL]-1,2-DIHYDRO-3H-DIBENZ[DE,H]-ISOQUINOLINE-1,3-DIONE S. SYNTHESIS, ANTITUMOR ACTIVITY, AND QUANTITATIVE STRUCTURE--ACTIVITY RELATIONSHIP. J Med Chem 1995;38(6):983-93.

Sets of 2-[2-(dimethylamino)ethyl]-1,2-dihydro-3H-dibenz[de,h]isoquinoline-1,3-diones with amino and actylamino groups at each of the eight positions on the anthracene nucleus were synthesized from appropriately substituted anthracenes. Their evaluation in in vitro antitumor and cardiotoxicity assays revealed a very strong dependence of potency on the position of substitution. Certain compounds, including the 4-, 5-, 7-, and 9-amino derivatives, showed significantly higher potency than the

unsubstituted parent compound, azonafide. Among them, 7-aminoazonafide had low cardiotoxicity relative to cytotoxicity. In general, the acetylamino analogues were less potent than the amino derivatives against tumor cells and neonatal rat heart myocytes; however, 5-(acetylamino)azonafide was highly cardiotoxic. 9-Aminoazonafide was more efficacious than azonafide or amonafide against P388 leukemia in mice. Statistically significant correlations were made between the ability of amino analogues to increase the transition melt temperature (delta Tm) of DNA and their potency against solid tumors, leukemia cells, or cardiac myocytes.

122
Da Silva CP, De Oliveira CR, De Lima MC. IN VITRO ASSAY OF THE EFFECTS OF ANTICANCER DRUGS ON LEUKEMIC T-CELLS BY FLOW MICRO-CALORIMETRY. Int J Pharm 1994;111(Oct 6):25-30.

The effects of some antineoplastic agents on leukemic T-cells were studied in vitro using flow microcalorimetry and conventional cell proliferation assays. Alterations in the metabolic heat power of cell suspensions due to drug treatment were measured as a function of time and dose of exposure and correlated with cell growth inhibition data. While no clear correlation was found for vincristine, a good correlation between calorimetric and cytotoxicity data was obtained for the other drugs. It was concluded that flow microcalorimetry may be used to examine the effects of antineoplastic agents on leukemic T-cells.

123
Morrison C, Macnair R, MacDonald C, Wykman A, Goldie I, Grant MH. IN VITRO BIOCOMPATIBILITY TESTING OF POLYMERS FOR ORTHOPEDIC IMPLANTS USING CULTURED
FIBROBALSTS AND OSTEOBLASTS. Biomaterials 1995; 16(13):987-92.

The biocompatibility of 2 polymers for potential use as orthopedic implant materials in an isoelestic hip prosthesis was investigated. The interactions of polyether ketone (PEEK) and epoxy resin polymers (with and without carbon fiber reinforcement) with both fibroblasts and osteoblasts were tested using cell protein, intracellular reduced glutathione (GSH), leakage of lactate dehydrogenase and the MTT assay as indexes of cellular cytotoxicity. The epoxy resin was slightly cytotoxic and inhibited the growth rate of fibroblasts (as assessed by total cell protein), and depleted GSH in both cell types. In contrast, the PEEK material did not display over signs of cytotoxicity and, in fact, increased osteoblast cell protein content. Of these 2 materials, PEEK would be the one of choice for development of an isoelastic implant and, in view of its stimulatory effect on osteoblast protein content, it may encourage ingrowth of bone around the prosthesis and thus minimize joint loosening.

124
Fulda S, Honer M, Menke-Moellers I, Berthold F. ANTIPROLIFERATIVE POTENTIAL OF CYTOSTATIC DRUGS ON NEUROBLASTOMA CELLS IN VITRO. Eur J Cancer 1995;31A(4):616-21.

The role of single drugs in the treatment of neuroblastoma is poorly defined.

Therefore, a monolayer proliferation assay was used to test neuroblastoma cell survival after a 72-h exposure to each of 20 cytostatic drugs. 6 Cell lines were selected on the basis of MYCN amplification and PGY1 overexpression. EC50 values were related to plasma levels achievable in patients during chemotherapy. The more effective substances were mitoxantrone, doxorubicin, hydroxyurea, bleomycin, dactinomycin, cisplatin, thiotepa, melphalan, carboplatin, etoposide, vincristine, cytarabine, 6-thioguanine, cyclophosphamide, ifosfamide and zilascorb. Parental drugs (e.g., cyclophosphamide, cisplatin) appeared to be more cytotoxic on a molar basis than derived drugs (e.g., ifosfamide, carboplatin). The least effective drugs were 5-fluorouracil, 6-mercaptopurine, CCNU and procarbazine. Fractional addn. of a given dose of cyclophosphamide, ifosfamide and cisplatin was more efficient than a single dose. The various neuroblastoma cell lines showed distinct individual sensitivities to the cytostatic drugs. Cell lines with MYCN amplification were more sensitive than PGY1-overexpressing cells. Thus, comparative in vitro testing of cytostatic drugs may provide a rationale for their clin. evaluation. Investigation of drug combinations and application of the monolayer proliferation assay to tumor biopsy material for preclin. chemosensitivity testing are clearly warranted.

125
Brueschweiler BJ, Wuergler FE, Fent K. CYTOTOXICITY IN VITRO OF ORGANOTIN COMPOUNDS TO FISH HEPATOMA CELLS PLHC-1 (POECILIOPSIS LUCIDA). Aquat Toxicol 1995;32(2,3):143-60.

Cytotoxicity in vitro to fish hepatoma cells PLHC-1 has been analyzed for a series of 21 organotin compds. consisting of all degrees of alkylation and arylation. The sensitivity of the neutral red (NR) assay and the tetrazolium salt redn. (MTT) assay was similar for most of the organotin compds. Cytotoxic effects were found at concns. between 10-8M and 10-2M For various trisubstituted organotin compds., including tributyltin and triphenyltin, which are used in antifouling paints and as pesticides, resp., cytotoxic concns. in the range of 10-8M to 10-6M were obsd. Based on the concn. reducing NR uptake by 50% (NR50), the sequence of cytotoxicity amongst butyltins was tributyltin > bis(tributyl)- tin > dibutyltin > tetrabutyltin > butyltin > tin(IV). Tributyltin induced effects on cell functions quickly, as a redn. in NR uptake by 30% was recorded after 30 min exposure to 4.cntdot.10-7M. The ranking order in cytotoxicity in the MTT assay of phenylated organotins was tri- phenyltin > diphenyltin > phenyltin > tin(IV). Cytotoxic concns. of tributyltin and triphenyltin measured in the bromodeoxyuridine (BrdU) assay and with the crystal violet (CV) staining method do not differ significantly from those detd. in the NR and MTT assay. Tri- and disubstituted organotin compds. exhibit a significant correlation between the n-octanol/water partition coeff. (logKow) and the NR50 (r = 0.86) and the MTT50 values (r = 0.85), resp. In addn., good qual. correlations between in vitro cytotoxicity data and in vivo fish toxicity data were found (for NR assay, r = 0.86,; for MTT assay, r = 0.80). The results indicate that the in vitro cytotoxicity assays using PLHC-1 cells are useful tools for the estn. of the acute toxicity to fish of organotins and possibly other compds.

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Kim HM, Oh GT, Han SB, Hong DH, Hwang BY, Kim YH, Lee JJ. COMPARATIVE EFFECTS OF ADRIAMYCIN AND 28-DEACETYL SENDANIN ON IN VITRO GROWTH INHIBITION OF HUMAN
CANCER CELL LINES. Arch Pharmacal Res 1994;17(2):100-3.

The limonoid compd. (28-deacetyl sendanin, I) isolated from the fruit of Melia toosendan SIEB. et ZUCC. was evaluated on anticancer activity. According to a std. in vitro cytotoxicity assay, eight human cancer cell lines and SRB assay were introduced for present evaluation. As a pos. std., adriamycin was tested in parallel. The cell lines were originated from six different organs. In view of dose-response profiles to 28-deacetyl sendanin, the most sensitive cells were SF-539 and PC-3 which were derived from CNS and prostate, resp. In contrast, all the cell lines responded similarly to adriamycin to give rise to nearly identical dose-response profiles. By comparison of GI50 between 28-deacetyl sendanin and adriamycin, six cell lines were more sensitive to 28-deacetyl sendanin and two were more resistant. As a result, 28-deacetyl sendanin had more sensitive and selective inhibitory effects on in vitro growth of human cancer cell lines in a comparison with adriamycin.

127
Lee MH, Kim CD, Ahn HJ. EVALUATION OF SURFACTANT CYTOTOXICITY POTENTIAL BY NEUTRAL RED UPTAKE ASSAY, MTT ASSAY AND CELL PROTEIN ASSAY. Korean J Toxicol 1994;10(2):215-20.

Ten surfactants were compared and ranked according to their cytotoxic potential. Cytotoxicity was measured by assessing lysosomal neutral red uptake, MTT redn. and protein measurement. The comparative cytotoxicity of surfactants, in decreasing order, shown by all test was cationics > anionics = amphoterics > nonionics. This is in agreement with in vivo observation. Surfactants also showed very similar rank order of toxicity with each of the asssay systems. Thus, these in vitro test methods may be considered reliable alternatives in the assessment of surfactant toxicity.

128
Gabor F, Wollmann K, Theyer G, Haberl I, Hamilton G. IN VITRO ANTIPROLIFERATIVE EFFECTS OF ALBUMIN-DOXORUBICIN CONJUGATES AGAINST EWING'S
SARCOMA AND PERIPHERAL NEUROECTODERMAL TUMOR CELLS. Anticancer Res 1994;14(5A):1943-50.

The 3-5 yr survival rates of patients with disseminated Ewing's sarcoma (ES) or the closely related peripheral primitive neuroectodermal tumors (PNET) remain low, even under aggressive treatment involving highly toxic multidrug chemotherapeutic regimens. ES and PNET are sensitive to doxorubicin, but may escape treatment by expression of the multidrug-resistant phenotype and/or other mechanisms. In this study, we have identified albumin as growth supporting factor for ES and PNET cells in IGF-I-supplemented serum-free tissue culture medium. To investigate the specificity and toxicity of albumin-based drug conjugates, doxorubicin was coupled to bovine serum albumin (BSA) by either a two step glutaraldehyde or carbodiimide-C4-spacer technique, yielding monomeric DOX-albumin conjugates with conjugation nos. ranging from 3

- 20 mol DOX/mol BSA. Cellular uptake of fluorescein-isothiocyanate-(FITC)-labeled albumin and DOX-albumin conjugates could be demonstrated by flow cytometric measurements of cell-assocd. fluorescence and confocal microscopy. The cytostatic activity of these conjugates against ES/PNET cell lines, a neuroblastoma (LAN-1) and prostate cancer carcinoma cell line (PC-3) and normal lymphoblasts was tested in short-term proliferation assays (48 h). The results show a high selectivity of the DOX-albumin conjugates for ES/PNET cell lines, with highest growth inhibition by conjugates with low DOX conjugation nos. (n = 3) in serum-supplemented medium (17 - 32 fold loss of activity compared to free DOX), followed by 20-DOX-C4-albumin in serum-free medium and low activity of the other conjugates. In conclusion, DOX-albumin conjugates inhibit the growth of ES/PNET cell lines selectively, showing low activity against the unrelated carcinoma line PC-3 and sparing normal lymphoblasts. The inverse correlation of activity and conjugation no. demonstrates a low cytotoxic activity of DOX in acid-stable binding to monomeric albumin, pointing to a selective cytostatic activity of the modified albumin against ES and PNET cells, even in the presence of a 100 fold excess of unmodified serum albumin.

129
Multhoff G, Meier T, Botzler C, Wiesnet M, Allenbacher A, Wilmanns W, Issels RD. DIFFERENTIAL EFFECTS OF IFOSFAMIDE ON THE CAPACITY OF CYTOTOXIC T LYMPHOCYTES AND NATURAL KILLER CELLS TO LYSE THEIR TARGET CELLS CORRELATE WITH
INTRACELLULAR GLUTATHIONE LEVELS. Blood 1995;85(8):2124-31.

We established an in vitro model to study the influence of ifosfamide treatment on intracellular glutathione (GSH) levels in activated human effector cells with specific phenotypes and immunol. functions. Besides its role as the major intracellular reductant, GSH has been shown to affect the initiation and progression of lymphocyte activation after stimulation with lectins. An incubation of activated human peripheral blood lymphocytes (PBL) with 4-hydroxyifosfamide, the activated form of ifosfamide (4-OH-IF), resulted in a depletion of the intracellular GSH levels and a significant inhibition of the proliferative capacity in a dose-dependent manner. The cytotoxic activity of sepd. CD3- natural killer (NK) cells and CD3+ allospecific, cytotoxic T lymphocytes (CTL), either untreated or treated with 4-OH-IF at different concns., was compared in a std. 51chromium release assay (CML). There were three major findings. (1) the capacity of CD3+ major histocompatibility complex (MHC)-restricted CTL to lyse their specific allogeneic target cells was substantially reduced by preincubation of the effector cells with 4-OH-IF. This inhibition of the lytic activity in CD3+ CTL correlated with a substantial depletion of the intracellular GSH levels in this population. Rapid reconstitution of depleted GSH levels and restoration of cytotoxic activity of CTL was achieved by incubation of the effector cells with thiols eg, glutathione ester (GSH-ester) or 2-mercapto-ethanesulfonate (mesna). (2) In contrast, the lytic activity in CD3- NK cells was not substantially affected (up to 100 mumol/L 4-OH-IF). This result correlates with the capacity of NK cells to maintain their intracellular GSH levels after an ifosfamide treatment. (3) In comparison with CD3+ CTL, CD3- NK cells are more resistant to ifosfamide treatment because they have higher initial GSH

levels and a more than fourfold higher relative rate of GSH synthesis.

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Joller PW, Coquette A, Noben J, Pirovano R, Southlee JA, Logemann PK. EUROPEAN INTERLABORATORY EVALUATION OF AN IN VITRO OCULAR IRRITATION MODEL SKIN-2 MODEL
ZK1100 USING 18 CHEMICALS AND FORMULATED PRODUCTS. In: Reinhardt, CA, editor. Alternatives to Animal Testing: New Ways in the Biomedical Sciences, Trends and Progress: Symposium; 1992 Nov 30; Zurich, Switzerland. New York, VCH Publishers, Inc; 1994. p. 159-64.

131

Berkers J AM, Hassing I, Spenkelink B, Brouwer A, Blaauboer BJ. INTERACTIVE EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND RETINOIDS ON PROLIFERATION
AND DIFFERENTIATION IN CULTURED HUMAN KERATINOCYTES: QUANTIFICATION OF CROSS-LINKED ENVELOPE FORMATION. Arch Toxicol 1995;69(6):368-78.

Dioxins are potent inducers of chloracne in humans. This skin aberration can be interpreted as an altered differentiation pattern of acinar sebaceous base cells and a change in the rate of terminal differentiation of the keratinocytes. We measured this rate induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in primary cultures of human keratinocytes. As parameters for differentiation, we quantified the 35S-methionine incorporation into cross-linked envelopes (revealing the total CLE biomass), as well as the number of microscopically visible CLEs. It was shown that TCDD is a very potent inducer of both CLE biomass and number with a half-maximal effect concentration (EC50) of 1.4 nM. CLE biomass was maximally increased 10-fold and the number of cells in culture producing a CLE was increased from 15% in control cultures to maximally 75% of the cells in TCDD-treated cultures. Both effects were Ca2+-dependent and increased with elevated cell density, being optimal in post-confluent cultures. Retinoic acid dose-dependently decreased the effect of 10-8 M TCDD, 10-6 M having a nearly complete antagonistic action. This interaction of retinoic acid with TCDD-induced differentiation was non-competitive. Retinol was equally potent as an antagonist of the TCDD-induced elevation of CLE formation as compared with retinoic acid. Retinyl palmitate and etretinate were not very effective as TCDD antagonists. Supplementation of hydrocortisone suppressed the TCDD-induced keratinocyte differentiation. It was concluded that CLE biomass quantification provides a reliable and sensitive parameter for keratinocyte differentiation. In this in vitro system it is shown that TCDD strongly induces a switch from proliferation to terminal differentiation and that this effect can be antagonized effectively by retinoic acid and retinol.

132

Frankild S, Anderson KE, Nielsen GD. EFFECT OF SODIUM LAURYL SULFATE (SLS) ON IN VITRO PERCUTANEOUS PENETRATION OF WATER, HYDROCORTISONE AND NICKEL. Contact
Dermatitis 1995;32(6):338-45.

The dose- and time-related effect of sodium lauryl sulfate (SLS) on in vitro percutaneous penetration was studied using 3 radiolabeled tracer compounds with different physicochemical properties: tritiated water, hydrocortisone and nickel. Human cadaver abdominal skin from Caucasian women was used as membrane in static in vitro penetration cells. Simultaneous application of SLS together with 1 of the tracer compounds showed, after 48 h, a significant dose-effect relationship between SLS concentration (0.25%, 2% and 10%) and penetration of tritiated water or nickel ($P<0.001$, Spearman), whereas SLS had no significant effect on penetration of hydrocortisone. When 4% SLS was applied as pretreatment, a significant time-effect relationship, after 48 h, was found between pretreatment time (0.5, 2 and 8 h) and penetration of tritiated water. A similar relationship was not found for penetration of nickel or hydrocortisone. Pretreatment of the skin with SLS for 2 h using 3 concentrations (0.25%, 4% and 10%) showed, after 48 h, a significant dose-effect relationship between SLS treatment and penetration of tritiated water or nickel ($P<0.001$, Spearman). Pretreatment had no effect on penetration of hydrocortisone. Pretreatment simulates a cleaning-washing situation. The present in vitro skin penetration model, using human cadaver skin, described the dose-effect and time-effect relationships for SLS on the penetration profiles of 3 different compounds. The model may be extended to other compounds with suspected irritant/damaging effect on the skin barrier. It should be kept in mind that the model uses a dead skin membrane without the barrier repair mechanisms of live skin.

133
Chang SK, Brooks JD, Monteiro-Riviere NA, Riviere JE. ENHANCING OR BLOCKING EFFECT OF FENVALERATE ON THE SUBSEQUENT PERCUTANEOUS ABSORBTION OF PESTICIDES
IN VITRO. Pestic Biochem Physiol 1995;51(3):214-9.

The percutaneous absorption of pesticides has been receiving much research attention. However, most work is conducted with single exposures and potential interactions of previous pesticide exposure have received little attention. In the present study, the effect of in vivo pretreatment of the skin with a 3% fenvalerate in ethanol or a 3% parathion in ethanol solution on carbaryl, fenvalerate, lindane, and parathion absorption was studied in vitro using weanling pig skin in a flowthrough diffusion cell system. Concentrations of 40 or 400 mug/cm2 of carbaryl, fenvalerate, lindane, and parathion in ethanol were applied topically. Environmental conditions of air and perfusate temperature (37~C), relative humidity (60%), flow rate (4 ml/hr), and Kreb's-Ringer bicarbonate buffer with 4.5% bovine serum albumin medium were controlled. The total absorption of these pesticides, both ethanol control and fenvalerate or parathion pretreated, increased proportionally with the dose; however, the absorption efficiency (fraction of applied dose absorbed) decreased as the dose increased. At both doses, fenvalerate pretreatment had little or no effect on carbaryl and fenvalerate absorption; however, parathion absorption was significantly decreased in fenvalerate-pretreated skin ($P < 0.05$). There was no significant difference ($P : 0.05$) of parathion absorption between the parathion pretreatment and the fenvalerate pretreatment. Lindane absorption increased at the 40-mug dose and significantly increased at the

400-mug dose (P < 0.05) following fenvalerate pretreatment. Carbaryl absorption was higher than other pesticides at the dose of 40 mug/cm2. Furthermore, comparing ethanol control data with previous results indicates that prolonged skin treatment with ethanol significantly increases parathion absorption (P < 0.05). This study suggests that the absorption data from a single parent compound alone were not adequate to determine the rate of absorption of pesticide under mixture exposure conditions. Pesticide interactions may significantly affect the percutaneous absorption, interpretation, and assessment of risk.

134
Alemohammad MM, Alki J, Foley TJ. DETECTION OF IgE ANTIBODIES TO LATEX ALLERGENS IN HUMAN SERUM. Contact Dermatitis 1995;32(5):298-302.

Reports indicate increasing incidence of Type I allergic reactions to latex allergens. The proteins that act as allergens and produce such allergic reactions are found in the natural latex sap of Hevea brasiliensis. All those who are exposed to latex products, particularly healthcare workers, are potentially at risk. The lack of qualified allergen extracts makes it difficult to perform skin testing on individuals who are at high risk. Therefore, a reliable in vitro test system for the detection of IgE antibodies to latex would be of considerable utility. We have developed a serological test for the qualitative determination of specific IgE antibodies to latex. In our study, 75 sera from individuals with a history of latex allergy and 29 serum samples from healthcare workers were tested by both radioimmunoassay (RIA) and enzyme immunoassay (EIA). The allergen bound to paper discs was the same solid phase for the RIA and EIA assays. The allergen preparation used for coating the paper discs was a mixture of proteins obtained from raw latex. The data show a good correlation between the results of RIA and EIA methods with data obtained using an RIA assay at an independent laboratory and by skin prick testing. Comparing the performance of our test using our latex material with that of the latex material obtained from the Food and Drug Administration (FDA), along with results of other tests including skin tests, we found that the specificity and sensitivity of our assay method approaches 100%. The data show no significant cross-reactivity between latex and banana. A low level of cross-reactivity between latex and avocado was observed. We conclude that, by using correct selection of proteins, the detection of specific IgE to latex may be a valuable assay method for screening individuals and for the diagnosis of allergy to latex.

135
Bonifer R, Neumann C, Meuer S, Schulze G, Herrmann F. INTERLEUKIN 5 EXPRESSING ALLERGEN-SPECIFIC T-LYMPHOCYTES IN PATIENTS WITH HOUSE DUST MITE
SENSITIZATION: ANALYSIS AT A CLONAL LEVEL. J Mol Med 1995;73(2):79-83.

Interleukin 5 (IL-5) is a T-cell lymphokine known to stimulate development, functional activity, and in vitro survival of eosinophils. Tissue and blood eosinophilia occurring during allergic responses of the immune system are potentially mediated by IL-5 secreting T-cells. To test this hypothesis a series of allergen-specific T-cell clones were established from peripheral

blood and skin lymphocytes of patients with atopic dermatitis and house dust mite sensitization. In addition, alloreactive T-cell clones were also

prepared from peripheral blood lymphocytes of healthy donors. Cloned T-cells were analyzed for IL-5 mRNA expression and IL-5 secretion by means of in vitro gene amplification using the reverse transcriptase polymerase chain reaction and IL-5 specific oligonucleotide hybridization, as well as IL-5-specific ELISA. A majority of allergen-specific long-term cultured T-cell clones (84%) of different donors and of either phenotype (CD8+ or CD4+) disclosed IL-5 transcripts on stimulation with lectins. Almost all clones exhibiting IL-5 transcripts also released immunoreactive IL-5 protein into their culture supernatants. In contrast, only 2% of alloreactive T-cell clones obtained from healthy donors and none of alloreactive T-cell clones of one atopic patient investigated expressed detectable amounts of IL-5 mRNA in response to lectin stimulation, all of whom were CD4+. These results suggest that eosinophilia observed in allergic responses in the peripheral blood and in tissues at the site of induced late-phase cutaneous reaction may be associated with IL-5 release by allergen-specific T-cells.

136
Dick D, Ng K ME, Sauder DN, Chu I. IN VITRO AND IN VIVO PERCUTANEOUS ABSORPTION OF 14C-CHLOROFORM IN HUMANS. Hum Exp Toxicol 1995; 14(3):260-5.

Chloroform has been found in potable water and there is concern that significant dermal absorption may arise from daily bathing and other activities. The present study examines percutaneous absorption of 14C-chloroform in vivo using human volunteers and in vitro using fresh, excised human skin in a flow-through diffusion cell system. Fifty microlitre doses of either 1000 mug ml-1 chloroform in distilled water, (16.1 mug cm-2) or 5000 mug ml-1 of chloroform in ethanol, (80.6 mug cm-1) were applied to the forearm of volunteers with exhaled air and urine being collected for analysis. Single doses of either 0.4 mug ml-1 chloroform in distilled water (low dose, 0.62 mug cm-2, 1.0 ml dosed) or 900 mug ml-1 chloroform in distilled water (high dose, 70.3 mug cm-2, 50 mul dosed) were applied to discs of the excised abdominal skin placed in flow-through diffusion cells and perfused with Hepes buffered Hank's balanced salt solution, with a wash at 4 h. In vivo absorption was 7.8 : 1.4% (water as vehicle) and 1.6 : 0.3% (ethanol as vehicle). Of the dose absorbed in vivo, more than 95% was excreted via the lungs (over 88% of which was CO2), and the maximum pulmonary excretion occurred between 15 min and 2 h after dosing. The percentage of dose absorbed in vitro (skin + perfusate) was 5.6 : 2.7% (low dose) and 7.1 : 1.4% (high dose). The above data demonstrate that a significant amount of the dissolved chloroform penetrates through the human skin, and that a higher percentage of the applied dose was absorbed using water as vehicle. In addition, the in vitro method offers a good estimate for in vivo data.

137
Chung JH, Youn JI. EFFECT OF ULTRAVIOLET A ON IL-1 PRODUCTION BY ULTRAVIOLET B IN CULTURED HUMAN KERATINOCYTES. J Dermatol Sci 1995;9(2):87-93.

Human skin is exposed to significant amounts of UVA and UVB radiation simultaneously. The effects of UVA and UVB interactions have been examined in many aspects such as erythema response. The effects of UVA on the production of cytokines by UVB have not been studied yet. The purpose of this study is to observe the effect of UVB and UVA on the production of IL-1 in cultured human keratinocytes and to determine whether UVA can modify the effects of UVB on the IL-1 production. Human keratinocytes derived from normal foreskin were exposed to UVA (0-30 J/cm2) and subsequently to UVB (0-50 mJ/cm2). After 48 h incubation, IL-1 levels in the culture supernatants and cell extracts of the cultured keratinocytes were measured by thymocyte proliferation assay. We have observed that UVB increased the production of IL-1 in cultured keratinocytes. However, UVA suppressed the production of IL-1 and also the stimulatory effect of UVB on the IL-1 production. We think that the opposite effects of UVB and

UVA on the IL-1 production in human keratinocytes might explain the different action mechanisms of UVB and UVA in many cutaneous responses.

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Edwards SM, Donnelly TA, Sayre RM, Rheins LA, Spielmann H, Liebsch M. CORRECTION OF PREVIEWS 97425447. QUANTITATIVE IN VITRO ASSESSMENT OF PHOTOTOXICITY USING A HUMAN SKIN MODEL, SKIN 2. ADDITION OF AUTHOR NAMES. Erratum published in Photodermatol Photoimmunol Photomed 1994;10(5):226. Photodermatol Photoimmunol Photomed 1994;10(3):111-7.

The ability to accurately predict the phototoxic potential of personal and skin care products remains a key element in assessing the safety of premarketed products. To find a reliable in vitro alternative test for photoirritancy, the European Commission and the European Cosmetic Association are conducting a 3-year, European validation study. Based on the results of this study, an in vitro photoirritancy method will be selected for incorporation into new international guidelines for photoirritancy testing. As a part of this study, Skin2, a cultured human skin system, was used to evaluate the phototoxic potential of chemicals with known photoirritative properties. The Skin2 ZH1351, a 3-dimensional co-culture system, consists of dermal fibroblasts and a multilayered epidermis comprising differentiated keratinocytes. This product line has previously been used to evaluate the irritative potential of topically applied ingredients and products. In this study, various concentrations of the test chemicals were applied to the epidermal side of the Skin2 tissue for contact times of 1 h or 24 h and then the tissue was exposed to 2.9 J/cm2 of ultraviolet A (UVA) radiation. Treated but nonirradiated tissues were also assayed to predict the cytotoxic potential of the test chemicals, which could mask the phototoxic reaction. After exposure, the tissue substrates were rinsed free of test chemicals and allowed to recover for 24 h. Following this incubation, the MTT reduction assay was used to assess cytotoxicity. The results of tests using this model skin substrate (Skin2) showed a high degree of correlation with data from human and animal models now used to evaluate the phototoxic potential of chemicals.

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French JE, Libbus BL, Hansen L, Spalding J, Tice RR, Mahler J, Tennant RW.

CYTOGENETIC ANALYSIS OF MALIGNANT SKIN TUMORS INDUCED IN CHEMICALLY TREATED TG
AC TRANSGENIC MICE. Mol Carcinog 1994;11(4): 215-26.

TG AC mice (which carry a v-Ha-ras transgene) rapidly develop papillomas in response to 12-O-tetradecanoylphorbol-13-acetate (TPA). Approximately 30% of the papillomas are associated with subsequent development of malignancies. Early-passage spindle-shaped tumor cells arising from explant cultures of TPA-induced tumors in TG AC mice were tumorigenic when transplanted to syngeneic recipients. The v-Ha-ras transgene in the transplanted tumors was expressed at a high level. To identify possible genetic changes associated with the development of malignant tumors, explanted cells were cultured in vitro and assessed for karyotypic changes between the second and third passages by analyzing C-banded metaphase chromosomes. For comparison, skin malignancies were induced in nontransgenic FVB/N mice (parent strain) by 7,12-dimethylbenz(a)anthracene (DMBA) initiation and TPA promotion, and their G-banded metaphase chromosomes were analyzed. Trisomy (in at least 50% of about 30 metaphases) of chromosome 15 (in five of 15 tumors) and chromosome 6 (four of 15 tumors) was observed in TGpendent of chemical treatment or tumor type. Of six tumors from DMBA/TPA-treated FVB/N mice, three had trisomy 10 or 15 (or both), and two appeared normal. The absence of trisomy 7 is notable because c-Ha-ras maps to that chromosome. The absence of trisomy 7 in the six FVB/N DMBA/TPA-induced skin malignancies contrasts with DMBA/TPA-induced karyotypic effects in SENCAR mice. Expression of the v-Ha-ras transgene may have precluded the requirement for endogenous mutant ras and allelic imbalance involving-chromosome 7 in TG/N mice. These results suggest the possibility that the observed trisomies are consequential, rather than causal, events in the development of TGgenetic analysis will be required to understand the changes associated with tumorigenesis in this transgenic line as well as in the parent mouse line.

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Hilton J, Kimber I. THE MURINE LOCAL LYMPH NODE ASSAY. Methods Mol Biol 1995;43:227-35.

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Supino R. MTT ASSAYS. Methods Mol Biol 1995;43:137-49.

142
Guan YB, Guo QZ, Zhang BZ. [TOXICOKINETICS OF UNSYMMETRICAL DIMETHYLHYDRAZINE
ABSORBED PERCUTANEOUSLY IN RABBITS.] Zhongguo Yaolixue Yu Dulixue Zazhi 1995;9(2):137-39. (Chi)

Blood concentration-time relationship was studied after percutaneous application (pc) of 78.2 and 156 mgbbit. The data were best fitted to a one-compartment open model with a first order absorption process. The half-lives (t1/2 ka) were 0.08 : 0.09 h and 0.19 : 0.12 h, respectively, while the fractions of absorption (f) were 0.11 : 0.04 and 0.17 : 0.09, respectively. Following subcutaneous injection (sc) of 78.2 mg kg-1 UDMH, its blood levels were much higher than those after percutaneous application

with an absorption rate f = 0.992, indicating almost complete absorption of the compound following sc. The t1/2 ka after sc was 0.68 : 0.19 h, which was significantly longer than those after pc. In vitro application of UDMH on rabbit skin demonstrated that 84.2 : 5.5% of the compound was evaporated from the skin, which might account for the low absorption rate of UDMH after pc in vivo. The elimination of UDMH was rapid, with a terminal elimination half-lives of 0.3-1.5 h. The amount of UDMH excreted in urine was 2.7%-18.9% of the total elimination, indicating that there might be elimination pathways of UDMH other than renal in rabbits.

143
Van de Sandt JJ, Rutten AA. DIFFERENTIAL EFFECTS OF CHEMICAL IRRITANTS IN RABBIT AND HUMAN SKIN ORGAN CULTURES. Toxicol In Vitro 1995; 9(2):157-68.

The toxicity of well known irritants was investigated in rabbit and human skin organ cultures. Test chemicals were selected from various categories of irritants and included both water-soluble and water-insoluble compounds. Using a highly standardized protocol, test chemicals were applied topically at concentrations relevant to the in vivo situation. Toxicity was assessed by histomorphological examination, inhibition of conversion of the tetrazolium salt MTT, inhibition of epidermal cell proliferation and release of pro-inflammatory hydroxy fatty acids. Chemicals that are known to induce irritation in vivo invariably caused histopathological changes and inhibition of cell proliferation, whereas non-irritating chemicals did not; however, inhibition of MTT conversion and release of hydroxy fatty acids occurred with only a limited number of irritants. The response to the chemical irritants was different in rabbit and human skin cultures. Rabbit skin was slightly more sensitive to sodium dodecyl sulfate and benzalkonium chloride than human skin. Moreover, mineral oil enhanced epidermal cell proliferation in rabbit skin, but not in human skin. This study demonstrates that chemical irritants cause substance- and species-specific effects in skin organ cultures. It is therefore unlikely that irritant potential of chemicals from all irritant categories can be detected in vitro using one single parameter. A multiple endpoint approach and the inclusion of human tissue are recommended for optimal in vitro irritancy testing of chemicals.

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Van de Sandt JJ, Maas WJ, Doornink PC, Rutten AA. RELEASE OF ARACHIDONIC AND LINOLEIC ACID METABOLITES IN SKIN ORGAN CULTURES AS CHARACTERISTICS OF IN
VITRO SKIN IRRITANCY. Fundam Appl Toxicol 1995; 25(1):20-8.

In vitro techniques make a major contribution to the development of alternatives to the in vivo "Draize" skin irritation test, and the development of sensitive and generally applicable in vitro endpoints of cutaneous toxicity is an area of intensive research. To investigate in vitro characteristics of cutaneous irritation, skin explants of rabbit and human origin were topically exposed to chemical irritants, after which the culture medium was analyzed for the presence of metabolites of both arachidonic and linoleic acid. In rabbits exposed to the potent irritant benzalkonium chloride, a direct relation was established between clinical signs of irritation and in vitro release of the

proinflammatory mediator 12-hydroxyeicosatetraenoic acid (12-HETE) by the exposed skin. Histological examination revealed varying degrees of epidermal damage. 12-HETE was also the predominant hydroxy fatty acid released in a dose-dependent way by rabbit skin cultures after in vitro exposure to sodium dodecyl sulfate (SDS), benzalkonium chloride (BC), and formaldehyde (FA). Human skin cultures released, in addition to 12-HETE, predominantly 15-HETE and 13-hydroxyoctadecadienoic acid (13-HODE), omega-6 oxygenase products of arachidonic acid and linoleic acid, respectively. The irritant-induced release of hydroxy fatty acids was strongly inhibited by the lipoxygenase inhibitor eicosatetraynoic acid, indicating enzyme-mediated generation of these bioactive lipids. Comparison of hydroxy fatty aci release to more established markers of cytotoxicity (leakage of the cellular enzymes, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH)) revealed that increased levels of 13-HODE, 9-HODE, 12-HETE, and ALT were specific markers of cutaneous irritancy in rabbit skin cultures. AST and LDH were more sensitive endpoints compared to ALT or hydroxy fatty acids, but failed to identify the difference in irritation capacity between SDS and BC reported in vivo. In human skin cultures, release of 12-HETE or 15-HETE occurred at irritant concentrations that did not result in enzyme leakage into the culture medium. In vitro effect levels of irritants were found comparable to the in vivo situation. We conclude that release of hydroxy fatty acids, in particular of 12-HETE, offers a mechanism-based characteristic of skin irritation which may be applicable to in vitro toxicity testing.

145
Pu Y, Bernstein IA. USE OF HUMAN PSEUDO-EPIDERMIS TO EVALUATE THE TOXICITY OF BIS-(2-CHOLROETHYL)SULFIDE (BCES) ON WATER PERMEATION BARRIER FORMATION AND
FUNCTION. Toxicol Let 1995; 76(1):85-91.

Human pseudo-epidermis was used to investigate the effects of bis-(2-chloroethyl)sulfide (BCES) on the formation and function of the water permeation barrier. To generate the culture, 2 million viable basal cells derived from human skin were plated on a Puropore nylon microporous membrane pre-coated with calf skin collagen. Addition of bovine pituitary extract and epidermal growth factor to the medium favored the formation of homogeneous cultures and better barrier function. The water permeation constant (Kp) was shown to decrease significantly and reached 25 : 6 from 71% of the cultures prepared. The effects of topically applied BCES on the incorporation of (14C)linoleic acid, as a marker for lipid synthesis, and Kp, as a measure of water permeation, were studied. Compared with untreated cultures, there was no difference in the Kp immediately after exposure to 1-10 nmol BCES/cm2 for 30 min. On the other hand, (14C)linoleic acid incorporation was dose-dependently decreased immediately after exposure and then returned to normal by 48 h later. These data suggest that BCES produces no direct damage to the water permeation barrier but may affect barrier formation by inhibiting lipid synthesis.

146
Norton JN, Rylander LA, Richards JL. IN VITRO ORAL MUCOSA IRRITATION TESTING

WITH HUMAN CELL CULTURES. Toxicol In Vitro 1995;9(1):67-74.

Cell cultures are a potentially useful model to predict in vivo oral mucosa irritation. To this end, oral mucosa organ equivalent cultures (OMOEC) and skin equivalent cultures (SEC), both derived from human tissue, were evaluated for their responsiveness to test dentifrices with graded degrees of irritation potential. OMOEC and SEC were treated with test dentifrices and responses were evaluated by histopathology, cell viability (MTT incorporation), and cytotoxicity (release of aspartate aminotransferase (AST)). Cell viability in OMOEC and SEC was reduced in a dose- and time-dependent manner in response to the test dentifrices. Correspondingly, AST release was increased in a dose- and time-dependent manner in response to the test dentifrices. These results demonstrate that OMOEC and SEC systems respond linearly to graded degrees of irritation potential as represented by generic dentifrices. Such results in an in vitro model of oral mucosa irritation allow direct comparison of in vitro responses with those obtained in an in vivo model, thus providing the groundwork for a tiered approach to assessment of irritation potential of oral care products.

147
Shieh HL, Hansen H, Zhu J, Riedel H. DIFFERENTIAL PROTEIN KINASE C LIGAND REGULATION DETECTED IN VIVO BY A PHENOTYPIC YEAST ASSAY. Mol Carcinog 1995;12(3):166-76.

The molecular dissection of protein kinase C (PKC) action has been based in part on time-consuming functional assays such as the mouse skin model for testing the tumor promoter activity of phorbol esters and related PKC activators. To help overcome the limitations imposed by the complexity of such assays, we developed the yeast Saccharomyces cerevisiae as an alternative, rapid, and simple experimental system. This model has a specific phenotype, an increase in the cell doubling time, that is proportional to the level of enzymatic activity of expressed mammalian PKC isoforms. We used this phenotype to assay and compare the regulation of native bovine PKCalpha and mutants in the conserved regulatory region C1 in vivo by various activators: two diterpenes, the phorbol ester phorbol-12-myristate-13-acetate (PMA) and mezerein, and the indole alkaloid indolactam V. We found that PMA activated PKC mutants lacking either Cys-rich, zinc finger-like repeat of the conserved region C1 to comparably reduced levels, whereas indolactam V activated native PKCalpha but none of the mutants at normal doses. In contrast, mezerein activated native PKCalpha and a mutant lacking the second Cys repeat equally well but mutants lacking the first Cys repeat of C1 at a greatly reduced level. These differential responses were supported by the observed in vitro PKC catalytic activities. Therefore, PMA regulates PKCalpha activity comparably well via either Cys repeat, whereas mezerein regulation predominantly occurs via the first Cys repeat of C1. Indolactam V activation was less potent, it was greatly reduced in the absence of either Cys repeat, and displayed no preference. We introduce this phenotypic assay as a rapid and general screen for the PKC-activating or possibly inhibitory potential of drug candidates and to identify the PKC regulatory sites involved in these interactions.

148
Vile GF, Tyrrell RM. UVA RADIATION-INDUCED OXIDATIVE DAMAGE TO LIPIDS AND PROTEINS IN VITRO AND IN HUMAN SKIN FIBROBLASTS IS DEPENDENT ON IRON AND SINGLET OXYGEN. Free Radic Biol Med 1995; 18(4):721-30.

This study describes the damage that occurs to lipids and proteins that have been irradiated in vitro or in human skin fibroblasts with physiological doses of UVA radiation. Thiobarbituric acid-reactive species were formed from hosphatidylcholine after UVA radiation in vitro. By using iron chelators, this process was shown to involve iron. Ferric iron associated with potential physiological chelators was reduced by UVA radiation, but iron within ferritin was not. By enhancing the half life-time with deuterium oxide or by using scavengers, singlet oxygen was also shown to be involved in the UVA radiation-dependent peroxidation of phosphatidylcholine. UVA radiation-generated singlet oxygen reacted with phosphatidylcholine to form lipid hydroperoxides, and the breakdown of these hydroperoxides to thiobarbituric acid-reactive species was dependent on iron. We have shown that iron and singlet oxygen are also involved in the UVA radiation-dependent formation of thiobarbituric acid-reactive species in human skin fibroblasts, and we propose that a similar concerted effect of iron and singlet oxygen is involved in UVA radiation-dependent damage to fibroblast lipids. Sulphydryl groups of bovine serum albumin and human gamma-globulin were oxidised upon UVA irradiation in vitro. The use of scavengers and deuterium oxide showed that UVA radiation-dependent sulphydryl oxidation was dependent on singlet oxygen. By adding or chelating iron, UVA radiation-dependent oxidation of sulphydryl groups of bovine serum albumin and human gamma-globulin was shown to be iron-dependent. The use of catalase and hydroxyl radical scavengers demonstrated that hydrogen peroxide, but not the hydroxyl radical, was involved. The oxidation of sulphydryl groups of proteins in human skin fibroblasts that occurs as a result of UVA irradiation was also shown to involve iron, singlet oxygen, and hydrogen peroxide. We conclude that iron, singlet oxygen, and hydrogen peroxide are important redox active species involved in the deleterious effects of UVA radiation on lipids and proteins of human skin cells.

149
Hertl M, Merk HF. LYMPHOCYTE ACTIVATION IN CUTANEOUS DRUG REACTIONS. J Invest Dermatol 1995;105(1 Suppl):95S-98S.

Peripheral blood lymphocytes from both drug-induced immediate and delayed cutaneous hypersensitivity reactions frequently can be stimulated in vitro with the particular culprit drug. Immunohistochemical analysis has identified CD8+ T cells as the predominant epidermal T-cell subset in drug-induced maculopapular and bullous eruptions and in patch-test reactions to beta-lactam antibiotics. Beta-lactam-specific peripheral and epidermal T lymphocytes from bullous exanthems were predominantly T-cell receptor alpha/beta+, CD8+, CD4-. Three CD8+ epidermal T-cell clones from penicillin-induced bullous exanthems displayed a TH1-like cytokine pattern and proliferated in an antigen- and major histocompatibility complex-specific manner. These epidermal T-cell clones were cytotoxic against autologous B cells upon stimulation through the T-cell receptor and against epidermal keratinocytes in lectin-induced

cytotoxicity assays. In contrast, peripheral T-cell lines from patients with penicillin-induced urticarial exanthems were predominantly T-cell receptor alpha/beta+, CD4+, CD8- and displayed a Th2-like cytokine pattern. CD8+ dermal T cells from a sulfamethoxazole-induced bullous exanthem proliferated in vitro in response to sulfamethoxazole. This T-cell proliferation was significantly increased in the presence of microsomes, which suggests that microsomal enzymes, such as cytochrome P450 enzymes, generate highly reactive metabolites which are the nominal antigens for T-cell activation. In summary, drugs may be processed and presented in different ways, which is reflected by the observation that Th1-like CD8+ T cells are primarily activated in delayed cutaneous hypersensitivity reactions, whereas Th2-like T-cell responses are present in patients with drug-induced urticarial exanthems.

150
Brenner S, Ruocco V, Wolf R, de Angelis E, Lombardi ML. PEMPHIGUS AND DIETARY FACTORS. IN VITRO ACANTHOLYSIS BY ALLYL COMPOUNDS OF THE GENUS ALLIUM. Dermatology 1995;190(3):197-202.

Today it is generally accepted that every drug that possesses an active thiol group in its molecule is capable of inducing pemphigus in vivo and provoking acantholysis in vitro. We therefore suggested that plants, in particular those belonging to the Allium group, that contain several active compounds with stable disulfide and thiol groups in their molecule may cause the same. OBJECTIVE: To verify this hypothesis by investigating the in vitro acantholytic effect of three compounds of garlic. METHODS: Skin samples from donors were cultured in the presence of three compounds of garlic (i.e. allylmercaptan, allylmethylsulfide and allylsulfide) for 3 days. The skin samples were then processed for microscopic control for acantholysis. RESULTS: Results indicate that, indeed, the three garlic compounds tested are capable of inducing acantholysis in vitro. Focal and diffuse acantholysis was observed in the specimens from 4 out of 7 donors cultured in the presence of 6 and 9 mM of each of the allyl compounds for 3 days. Interestingly, tissues from a DR4+ donor proved to be more acantholysis prone than others, showing large blistering due to diffuse acantholysis, thus indicating that individual susceptibility plays a crucial role also in vitro. CONCLUSION: Garlic compounds with stable disulfide and thiol groups in their molecule are capable of inducing acantholysis in vitro. These findings lend further support to the theory that 'harmless' nutritional factors are capable of inducing acantholysis in vitro and possibly also in vivo. In view of these findings, it is suggested that nutritional factors should be added to the ever-growing list of exogenous factors capable of inducing pemphigus.

151
Dominguez MC, Sole E, Goni C, Ballabriga A. EFFECT OF ALUMINUM AND LEAD SALTS ON LIPID PEROXIDATION AND CELL SURVIVAL IN HUMAN SKIN FIBROBLASTS. Biol Trace Elem Res 1995;47(1-3):57-67.

The aim of this study was to see whether aluminum (Al) and lead (Pb) salts are toxic for cultured human fibroblasts under different experimental conditions, in the controllable situation offered by cell cultures. Cell survival and membrane lipid peroxidation served as markers of Al and Pb toxicity.

Evaluation of the living cells was carried out using a colorimetric method, the mitochondrial reduction of 1-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Lipoperoxidation assay was performed on whole cell homogenates by measuring thiobarbituric acid-reactive substances (TBARS) produced after incubation with ascorbic acid-ferrous sulfate. Al(III) and Pb(II) salts (300 microM) produce a considerable decrease in cell survival after an exposure period of 4d, evident with the three fetal calf serum concentrations in the culture media: 2, 5, and 10%. Taking into account in vitro cell aging, the cytotoxic effects of Al(III) and Pb(II) are greater in senescent fibroblasts than in young cells. Lead-induced cytotoxicity is higher than Al-induced cytotoxicity. A mechanism that contributes to cellular toxicity is membrane lipid peroxidation; our results demonstrate that Al(III) and Pb(II) ions, 400 microM, exert an antioxidant-like effect or a pro-oxidant action on cell membranes depending on exposure time. We describe significant increases in TBARS formation associated with the presence of 400 microM Al(III) or Pb(II) salts in the culture media. Our study also revealed that these heavy metals induce a cell age-dependent action on membrane lipoperoxidation that is greater in senescent fibroblasts and this could have severe consequences for maintenance of cellular integrity.

152
Fullerton A, Menne T. IN VITRO AND IN VIVO EVALUATION OF THE EFFECT OF BARRIER GELS IN NICKEL CONTACT ALLERGY. Contact Dermatitis 1995;32(2):100-6.

The protective effect of various ethylenediaminetetraacetate (EDTA) barrier gels on nickel skin penetration was investigated in an in vitro model using human skin. Application of the gels seemed to cause an increased release of nickel from nickel alloys. This nickel did not penetrate the skin barrier but was found to be immobilized on the skin surface. This emphasized the importance of washing the skin surface to remove any surplus of barrier formulation after use, since considerable amounts of nickel will be bound in this formulation. It was found that application of the barrier gels beneath the nickel alloy in contact with the skin significantly reduced the amount of nickel found in the epidermal skin layer. In vivo patch testing with a disc of nickel alloy, with and without use of barrier gel, was performed in 21 nickel-sensitive patients. Patch testing with the nickel alloy without use of barrier gel resulted in positive patch test reactions in 11/21 (52.4%) of the patients tested. Application of a Carbopol gel with 10% CaNa2-EDTA beneath the nickel disc completely abrogated the allergic contact response in all 21/21 (100%) patients. A Carbopol gel without CaNa2-EDTA was less effective, inhibiting the response in 15/21 (71.4%). A high concordance was found between epidermal nickel levels found in vitro and the in vivo patch test.

153
Farriol M, Mourelle M, Schwartz S. EFFECT OF VITAMIN C AND VITAMIN E ANALOG ON AGED FIBROBLASTS. Rev Esp Fisiol 1994;50(4):253-7.

Human dermal fibroblasts were cultured and aged in vitro. Survival of young and aged fibroblasts was determined in the presence and absence of different concentrations of two vitamins. Vit C at doses of 5, 12.5, 25 and 50 mumol/L and water-soluble Vit E (Trolox) at 1, 5, 10 and 50 mg/L, were added 30

minutes before oxidative stress, consisting of exposure to 5 mM hydrogen peroxide for 30 minutes. A non-radioactive cell proliferation cytotoxicity assay (MTT) was used to determine the protective effect of the vitamins studied. Vit C produced a clear cytoprotective effect on aged cells over the entire range of doses applied. The protection provided by Vit E, was less pronounced.

154
Fabreguette A, Zhi Hua S, Lasne F, Damour O. [EVALUATION OF THE CYTOTOXICITY OF ANTISEPTICS USED IN CURRENT PRACTICE ON CULTURES OF FIBROBLASTS AND KERATINOCYTES.] Pathol Biol 1994; 42(9):888-92. (Fre)

Infection is the greatest problem in burn patients and topical antiseptics must be chosen with great care especially when cultured skin is grafted. We examined the cytotoxicity of 6 antiseptics commonly used on cultured human fibroblasts and keratinocytes. Cultured cells were exposed for 15 min to Hibitane (chlorhexidine), Biseptine (chlorhexidine + benzalkonium chloride + benzylic alcool), dermic Betadine (polvidone iodine + nonoxinol), scrub Betadine (polyvidone iodine + quaternary ammonium) and gynecologic Betadine (polyvidone iodine). The cell viability was determined using the MTT test. At therapeutic concentration all the antiseptics were cytotoxic for fibroblasts and keratinocytes. The data suggest that the antiseptics must be used in function of the time of the grafting of the cultured epithelium.

155
Moody RP, Nadeau B, Chu I. IN VIVO AND IN VITRO DERMAL ABSORPTION OF BENZO[A]PYRENE IN RAT, GUINEA PIG, HUMAN AND TISSUE-CULTURED SKIN. J Dermatol Sci 1995;9(1):48-58.

Cross-species in vitro dermal absorption tests were conducted with 14C-labelled benzo[a]pyrene dissolved in acetone and applied to dermatomed skin (0.5 mm thickness) at comparable dose rates (8-13 micrograms/cm2). Skin absorption was determined using the Bronaugh in vitro flow-through procedure. The percentage (%) dermal absorption included the % 14C-activity detected persisting in the skin added to that detected in the receiver solution. Listed in decreasing order, total % in vitro dermal absorption obtained by 48 h postexposure was: 95 +/- 9.6% (rat), 51 +/- 3.0% (hairless guinea pig), 43 +/- 8.7% (human; 50-year-old), 34 +/- 12.4% (Testskin) and 23 +/- 5.3% (human; 32-year-old). Comparative in vivo studies demonstrated urinary recovery of 8 +/- 1.8% and 25 +/- 5.0% for rats (dose rate: 6 micrograms/cm2) and hairless guinea pigs (dose rate: 9 micrograms/cm2), respectively. Total faecal recovery was 61 +/- 6.0% and 43 +/- 6.1% for rats and guinea pigs, respectively. Necropsies conducted at 14 days postexposure demonstrated total 14C-activity tissue recoveries of 0.5 +/- 0.13% and 0.6 +/- 0.17% in rats and guinea pigs, respectively. Including the 14C-activity extracted from the skin removed from the dose site at 14 days postexposure, the total % in vivo dermal absorbtion was 70 +/- 7.6% and 68 +/- 9.3% for rats and guinea pigs, respectively. In summary, the in vitro data was consistent with the in vivo data in demonstrating that 14C-benzo[a]pyrene was well absorbed through skin.

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Silvennoinen-Kassinen S, Vainio O, Karvonen J, Kauppinen M, Kallioinen M. ADHESION MOLECULES IN THE NICKEL ALLERGIC REACTION. Int Arch Allergy Immunol 1995;106(4):345-50.

Nickel is the major cause of allergic contact dermatitis, and to increase our understanding of this immune reaction we studied changes in the expression of adhesion molecules on mononuclear cells during nickel stimulation in vivo and in vitro. Nickel-induced lymphocyte cultures were used in vitro, the cells being examined with monoclonal antibodies (Mabs) and by flow cytometry. Mononuclear cells from skin biopsies of in vivo cutaneous nickel reactions were studied with Mabs and immunohistochemistry. The expression of adhesion molecules in vitro was differential: the number of cells carrying CD11c, CD29, CDw49b, CDw49d, CDw49e, CDw49f, CD54, CD56 and ELAM-1 being significantly overrepresented among the nickel-induced lymphoblasts whereas the number of blasts carrying CD44 was underrepresented and those of CD11a, CD18, CD58 and LAM-1 remained unchanged. CD4+ cells gained adhesion molecules during nickel-induced blast transformation whereas CD8+ cells lost most of their adhesion molecules. The in vivo results were in agreement with the in vitro ones except that CDw49b, CDw49f, CD56 and ELAM-1 could not be detected in a 96-hour nickel reaction in vivo. In conclusion, the nickel allergic reaction favors the expression of certain adhesion molecules, and this expression is induced on CD4+ cells while CD8+ cells tend to lose such molecules. The changes were more sensitively detected with the in vitro method.

157
Burnet NG, Nyman J, Turesson I, Wurm R, Yarnold JR, Peacock JH. THE RELATIONSHIP BETWEEN CELLULAR RADIATION SENSITIVITY AND TISSUE RESPONSE MAY
PROVIDE THE BASIS FOR INDIVIDUALISING RADIOTHERAPY SCHEDULES. Radiother Oncol 1994;33(3):228-38.

There is a wide variation in normal tissue reactions to radiotherapy and in many situations the severity of these reactions limits radiotherapy dose. Clinical fractionation studies carried out in Gothenburg have demonstrated that a large part of the spectrum of normal tissue reactions is due to differences in individual normal tissue sensitivity. If this variation in normal tissue reactions is due to differences in intrinsic cellular radiosensitivity, it should be possible to predict tissue response based on measurement of cellular sensitivity. Here we report the initial results of a study aimed at establishing whether a direct relationship exists between cellular radiosensitivity and tissue response. Ten fibroblasts strains, including four duplicates, were established from a group of patients in the Gothenburg fractionation trials who had received radiotherapy following mastectomy. Skin doses were measured and both acute and late skin changes were observed following radiotherapy. Right and left parasternal areas were treated with different dose fractionation schedules. Clonogenic assays were used to assess intrinsic cellular radiosensitivity, and all experiments were carried out without prior knowledge of the clinical response, or which strains were duplicates. Irradiation was carried o t using 60Co gamma-rays at high dose-rate (HDR) of 1-2 Gy/min and low dose-rate (LDR) of 1 cGy/min. A spectrum of sensitivity was seen, with SF2 values of 0.17-0.28 at HDR and 0.25-0.34 at

LDR, and values of D0.01 of 5.07-6.38 Gy at HDR and 6.43-8.12 Gy at LDR. Comparison of the in vitro results with the clinical normal tissue effects shows a correlation between cellular sensitivity and late tissue reactions, which is highly significant with p = 0.02. A correlation between cellular sensitivity and acute effects was noted in the left-sided parasternal fields, but not the right. This is thought to be coincidental, and without biological significance. Our results suggest that cellular sensitivity might form the basis for the development of an assay system capable of predicting late normal tissue effects to curative radiotherapy, which might allow dose escalation in some patients. Increased local control and cure, with unchanged or improved normal issue complications, could result from such individualised radiotherapy prescriptions.

158
Chang SK, Brownie C, Riviere JE. PERCUTANEOUS ABSORPTION OF TOPICAL PARATHION THROUGH PORCINE SKIN: IN VITRO STUDIES ON THE EFFECT OF ENVIRONMENTAL PERTURBATIONS. J Vet Pharmacol Ther 1994;17(6):434-9.

Topical use of pesticides in domestic animals such as swine is a common practice; however, the effect of environmental factors on the extent of absorption has not ceived attention. Since no single factor can exert its effects alone in the natural environment, the interaction of environmental factors on the percutaneous absorption of pesticides must be understood before potential toxicity of dermal absorption of pesticides can be effectively estimated. In the present studies, the effects of air temperature (Ta), perfusate temperature (Tp), perfusate flow (F) and relative humidity (%RH) on absorption of parathion were studied in vitro in porcine skin. Parathion absorption was determined by measuring radiolabel appearing in the perfusate over time. Three main environmental parameters were found to have a significant effect on parathion penetration. Increasing Ta from 37 degrees C to 42 degrees C, %RH from 60% to 90% or F from 4 ml/h to 8 ml/h each produced a significant increase in penetration. The following significantly positive two-way interactions among test parameters were seen: Ta x F and %RH x F at the 4 micrograms dose, %RH x F at the 40 micrograms dose and Ta x %RH, Ta x F and %RH x F at the 400 micrograms dose. There were no three-way interactions at any of the three doses tested. These results suggest that the factors tested are not independent variables and must be considered interactive when used in assessing pesticide percutaneous absorption.

159
Vock EH, Cantoreggi S, Gupta RC, Lutz WK. 32P-POSTLABELING ANALYSIS OF DNA ADDUCTS FORMED IN VITRO AND IN RAT SKIN BY METHYLENEDIPHENYL-4,4'-DIISOCYANATE
(MDI). Toxicol Lett 1995;76(1):
17-26.

The 32P-postlabeling method was adapted for the detection of DNA adducts formed by methylenediphenyl-4,4'-diisocyanate (MDI). Incubation of the 3'-phosphates of the deoxyribosides of cytosine (C), adenine (A), guanine (G) and thymine (T) with MDI in Tris buffer resulted in the formation of 5, 7, 8, and 2 reaction products, respectively. Incubation of DNA with MDI resulted in

detectable levels of 5, 2, and 1 adducts attributable to C, A, and G. Analysis of DNA isolated from the epidermis of rats treated dermally with 9 mg MDI showed an adduct pattern similar to the one seen in the in vitro DNA incubation. A total adduct level of 7 per 10(8) nucleotides was measured, the limit of detection was 2 adducts per 10(10) nucleotides. The data indicate that a minute fraction of MDI can reach DNA in vivo in a chemically reactive form. In comparison with the genotoxic skin carcinogen 7,12-dimethylbenz[a]anthracene on the other hand, the DNA-binding potency of MDI was more than 1000-fold lower.

160
Liu C, Ho H, Hsieh M, Sokoloski TD, Sheu M. STUDIES ON THE IN VITRO PERCUTANEOUS PENETRATION OF INDOMETHACIN FROM GEL SYSTEMS IN HAIRLESS MICE. J
Pharm Pharmacol 1995;47(5):365-72.

The influence of co-solvents on the in-vitro percutaneous penetration of indomethacin from gel systems was studied using a simplex lattice exptl. design. Gel formulations were prepd. by gelling the vehicle mixt. of water, either alc. or isopropanol and either propylene glycol or PEG 400 with 1% wt./wt. Carbomer 940. Hairless mouse skin was employed as the barrier in a Franz-type diffusion cell. The penetration rates at steady state for seven formulations were fitted to a polynomial equation based on this simple lattice method and a three-dimensional plot was constructed. The formulation having the maximal penetration rate was detd. to be the vehicle with a solvent ratio of water/alc./propylene glycol equal to 15:33:52, and which possessed a soly. parameter. For those vehicles with a soly. parameter <15, both the drug soly. and the penetration rate decreased with a decrease in the soly. parameter. There was shown to be an approx. 20-fold increase in the relative enhancement factor when using both alc. and isopropanol, but only a threefold increase for both propylene glycol and PEG 400, when compared with water.

161
Carter WG, Gil SG, Ryan MC. IN VITRO MODEL SYSTEM FOR STUDY OF BASAL (STEM) CELL FUNCTION INVOLVING FUNCTIONAL EPILIGRIN DETECTION. PCT Int. Appl. PATENT NO. 95 06660 03/09/95 (Fred Huchinson Cancer Research Center).

An in vitro epithelial model system where basal (stem) cells may be studied is presented. Previous to this work, studies of basal cell interactions with basement membranes has been complicated due to alterations in structure, shape, and compn. of basement membranes during development and acquisition of specialized cellular functions. The assay presented here circumvents these problems. Epiligrin is the major adhesion ligand present in the epidermal basement membrane and mediates basal cell adhesion through integrin alpha3beta1 in focal adhesions and alpha6beta4 in hemidesmosome adhesion structures. CDNA sequences encoding the E170 epithelial ligand are presented and may be used in the above assay. These sequences are useful in expressions systems and may be used as diagnostic and therapeutic agents in identifying and treating patients with the gravis form of junctional epidermolysis bullosa and other epithelial diseases, including inflammation. Further, methods are provided for the purifn. and utilization of this glycoprotein and for raising

antibodies against of this complex. Methods for identification of functional epiligrin in tissues are also given.

162
Ahmed S, Imai T, Otagiri M. STEREOSELECTIVE HYDROLYSIS AND PENETRATION OF PROPRANOLOL PRODRUGS: IN VITRO EVALUATION USING HAIRLESS MOUSE SKIN. J Pharm
Sci 1995;84(7):877-83.

Stereoselective hydrolysis of two ester prodrugs of propranolol, isovaleryl propranolol (IV-PL) and cyclopropanoyl propranolol (CP-PL), was studied in Tris-HCl buffer (pH 7.4) contg. 0.15 M KCl, skin and liver homogenates, 5% plasma in Tris-HCl buffer, skin cytosol and microsomes, and liver cytosol and microsomes. The hydrolysis rate consts. of (R)-isomers of the prodrugs were 1.1-30.3 times greater than those of the resp. (S)-isomers in tissue prepns. Skin showed considerable metabolic activity and very high stereoselectivity (R/S ratio: 7.3-30.3). The hydrolyzing capacities of buffer and different tissue prepns. per mg of protein content were in the following increasing order: buffer < skin homogenate < plasma < liver homogenate. The studies with microsomes and cytosols indicated that the esterases, which are responsible for the hydrolysis of prodrugs, were mainly present in the cytosic and microsomal fractions of skin and liver, resp. There was a good correlation between the octanol-buffer partition coeffs. of propranolol and its prodrugs and the skin partition coeff. In vitro stereoselective penetration of propranolol and the prodrugs through full-thickness hairless mouse skin was evaluated with flow-through diffusion cells. Although the concn. of propranolol was 14-22 times greater than those of the prodrugs in the donor chamber, the steady-state flux of propranolol isomers [10.72 and 10.64 ug/cm2.cntdot.h for (R)- and (S)-isomers, resp.] were similar to those of CP-PL [10.80 and 10.78 mug/cm2.cntdot.h for (R)- and (S)-isomers, resp.] and even lower than those of IV-PL [14.51 and 14.33 mug/cm2.cntdot.h for (R)- and (S)-isomers, resp.]. Moreover, the permeability coeffs. of IV-PL [2.82 .times. 10-3 and 2.78 .times. 10-3 cm/h for (R)- and (S)-isomers, resp.] and CP-PL (1.29 .times. 10-3 cm/h for each isomer) were 14-30-fold greater than those of propranolol isomers (0.09 .times. 10-3 cm/h for each isomer). The diffusion coeffs. of all the compds. were similar, but their solvent membrane distribution coeffs. differed greatly and proved that the higher permeability coeffs. of the prodrugs were due to the higher affinity of the prodrugs for skin. Neither propranolol nor the prodrugs showed stereoselective penetration. However, highly stereoselective hydrolysis occurred during penetration of the prodrugs, and the R/S ratios of the cumulative amt. of delivered propranolol in 12 h were 11 and 13 for IV-PL and CP-PL, resp. A skin irritation test was performed in Japanese white male rabbits and no irritation was obsd. In conclusion, the hairless mouse skin possesses highly stereoselective esterase activity, and IV-PL and CP-PL might be promising prodrugs for transdermal delivery of higher amts. of drug from a much lower initial concn. compared with propranolol.

163
Plate H. MAGNESIUM SURFACTANTS. CLEANSING AT ITS BEST AND MILDEST. Parfuem Kosmet 1995;76(1):28-32.

The application of Mg contg. surfactants in cosmetics and toiletries for mild formulations is described. Comparison of Mg fatty alc. sulfates and ether sulfates with Na analogous for skin compatibility and application properties showed a low irritation potential for Mg types of surfactants in several in-vitro and in-vivo tests. Investigations of cleansing, foaming, and dermatol. properties showed favorable results for Mg surfactants, which fulfill all requirements to become a major surfactant of cosmetics and toiletries.

164
Turowski A, Skrypzak W, Reng A, Juerges P. [CHARACTERIZATION OF MILD SURFACTANTS FOR HAIR AND SKIN-CLEANSING PRODUCTS.] Parfuem Kosmet 1995;76(1):16-27. (Ger)

A reveiw describing environmental friendly mild surfactants in cosmetic formulations, esp. for hair and skin-cleansing prepns. with less irritation potential, including alkyl ether sulfates, amide ether sulfates, alkyl amidopropyl-betaines, alkyl ether carboxylic acids, sulfosuccinic acid ester, ampho(di)acetate, fatty acid condensation products, and alkyl polyglycosides. The synthesis route of some of these surfactants are described. The in vitro methods for evaluation of irritation potentials to skin including zein/isopropyl myristate protein solubilization testing, red blood cell test, hemolysis test, Hb denaturing, and hen's egg chloroallantoic membrane test are described.

165
European Centre for Ecotoxicology and Toxicology of Chemicals. SKIN IRRITATION AND CORROSION: REFERENCE CHEMICALS DATA BANK. Tech Rep - ECETOC 1995;(66):247
pp.

Earlier ECETOC (Europen Center for Ecotoxicol. and Toxicol. of Chems.) has published comprehensive listing of in vivo rabbit eye irritation data for 55 readily-available chems. of high purity. The establishment of such a data bank allows investigators of in vitro or alternative methods to evaluate their own techniques without the need to carry out in vivo testing of the ref. chems. A companion data bank has now been developed for 176 chems. for which comprehensive rabbit skin irritation/corrosion data are available. No new in vivo testing has been carried out to qualify a chem. for inclusion in this list. The 176 chems. selected are readily available at high and consistent purity and are expected to be stable on storage. They have been tested undiluted in in vivo studies, excepting those chems. where high concns. of the substance could be expected to cause severe effects. The in vivo data have been generated since 1981 in studies carried out according to OECD Test Guideline 404 and following the principles of Good Lab. Practice. The data presented were obtained from tests normally using at least three rabbits, involving application of 0.5mL (or 0.5g) to the flank under semi-occlusive patches and in which observations were made at least 24, 48, and 72 h after application. The chems. represent a range of chem. classes (acids, acrylates/methacrylates, alcs., aldehydes, alkalis, amides, amines, brominated

derivs., chlorinated solvents, esters, ethers, fatty acids and mixts. fragrance oils, halogenated aroms., hydrocarbons (unsatd.), inorgs., ketones, nitriles, phenolic derivs., S-contg. compds., soaps/surfactants, triglycerides) and different degrees of irritancy. The chems. are ranked for skin irritation potential on the basis of a 'primary irritation index'. They should be of use in validation tests of promising alternatives to the in vivo rabbit skin irritation/corrosion test. This is an essential step in the progression to regulatory acceptance. Classification schemes for chems. on the basis of their skin irritation/corrosion properties are appended to the report for the convenience of readers.

166
Lalor CB, Flynn GL, Weiner AN. FORMULATION FACTORS AFFECTING RELEASE OF DRUG FROM TOPICAL VEHICLES. II. EFFECT OF SOLUBILITY ON IN VITRO DELIVERY OF A SERIES OF N-ALKYL P-AMINOBENZOATES. J Pharm Sci 1995; 84(6):673-6.

The major influence on the rate of drug transfer out of its vehicle and into the skin is the thermodn. activity of the drug within its formulation. This study addresses certain thermodn. dependencies of topical delivery in a model system. Prototypical water-in-oil (W/O) and oil-in-water (O/W) emulsions and their component phases are used as the test vehicles, polydimethylsiloxane is the membrane, and three homologous n-alkyl p-aminobenzenzoate esters are the test permeants. In an emulsion, the interaction of the compd. between the water and oil phase can det. the extent of lowering of the thermodn. activity in the external phase in contact with the membrane. The emulsifiers (surfactants) impact strongly on partitioning and permeation as a result of the extra solubilizing capacity contributed by the surfactant micelles. The lower flux in the aq. phase of the O/W emulsion is the result of micellar solubilization, and this solubilization increased with increasing ester chain length. Solubilization is also an influence in nonaq. phases, but permeant hydrophobicity is without specific influence; therefore, transport becomes less dependent upon the structure of the compd.

167
Amdidouche D, Montassier P, Poelman MC, Duchene D. EVALUATION BY LASER DOPPLER VELOCIMETRY OF THE ATTENUATION OF TRETINOIN INDUCED SKIN IRRITATION BY
BETA-CYCLODEXTRIN COMPLEXATION.
Int J Pharm 1994;111(Oct 20 1994):111-6.

The release of free tretinoin and its inclusion complex with beta-cyclodextrin from a dermic gel was studied in vitro using Franz diffusion cells, and skin irritation induced by the preparations was studied in 9 healthy subjects (mean age 27 yr) using laser Doppler velocimetry. The in vitro study showed the release of free drug and its inclusion complex from the gels. Eight h after beginning the diffusion test, release rates were 10% for included drug and 20% for free drug. Skin irritation was significantly reduced on complexation. The attenuation of the irritation induced by including the drug in beta-cyclodextrin was greater than 85%. It was concluded that laser Doppler velocimetry may be used to assess skin irritation induced by a complex of tretinoin and beta-cyclodextrin.

168
Schrader K. [PHYSIOLOGICAL EVALUATION OF SURFACTANTS IN CONTACT WITH THE SKIN.] Parfuem Kosmet 1994;75(Feb):68-85. (Ger)

A series of in vivo and in vitro test procedures to guarantee the physiological compatibility of surfactants and their blends as raw materials of detergents and cleaning agents with the skin surface are presented; the results obtained by these methods are also discussed.

169
Collier SW, Sardon S, Ruiz-Cabello J, Johnson WA, Schwartz SL, et al. MEASUREMENT OF PHARMACODYNAMIC EFFECTS OF DEXAMETHASONE ON EPIDERMIS BY PHOSPHORUS NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY IN VITRO. J Pharm Sci 1994;83(Sep):1339-44.

To assess topical corticosteroid bioequivalence, pharmacodynamic effects of varying doses of dexamethasone acetate (dexamethasone-21-acetate) on perfused, intact, viable swine epidermis were evaluated by measuring intracellular phosphorus metabolism with nuclear magnetic resonance spectrometry. Dexamethasone perfusion resulted in a biphasic effect on phosphomonoester metabolism and a dose-dependent decrease in phosphocreatinine and nucleotide triphosphate levels. In addition, log-linear relationship was observed between dexamethasone dose and a decrease in phosphocreatinine. It was concluded that these techniques may be useful for elucidating mechanisms of corticosteroid action on the skin and may serve as a basis for developing a relevant bioequivalence technique.

170
Reifenrath WG, Lee B, Wilson DR, Spencer TS. COMPARISON OF IN VITRO SKIN-PENETRATION CELLS. J Pharm Sci 1994;83(Sep):1229-33.

A low volume flow-through diffusion cell that determines penetrant flux across skin while minimizing the dilution of penetrant in receptor fluid and eliminating the need for magnetic stirring was compared to a high volume flow cell and a magnetically stirred, manually sampled static cell with hydrophilic and lipophilic penetrants. Results indicated that percutaneous absorption and residue of tested drug did not differ between the low volume and high volume diffusion cells. In addition, low volume diffusion flux profiles accurately represented those of the static cell.

171
Su MH, Srinivasan V, Ghanem AH, Higuchi WI. QUANTITATIVE IN VIVO IONTOPHORETIC STUDIES. J Pharm Sci 1994;83(Jan):12-7.

An experimental method was developed to evaluate in vitro-in vivo correlations in iontophoretic drug delivery and the method was used to examine the release of tetraethylammonium bromide (tetrylammonium bromide) from an iontophoretic device in vitro and its iontophoretic delivery in mice with and

without permeation enhancers. Good in vitro-in vivo correlation was found when the dominant barrier to transport was the same in both situations. Blood levels could be predicted using permeability coefficients and elimination parameters, provided that iontophoretic flux was the same at the same current density, skin metabolism and adsorption effects were insignificant, and elimination kinetics were not significantly affected. It was concluded that the method may be used to evaluate the efficacy of a permeation enhancer used with iontophoresis.

172
Barthes D, Bosset M, Rochard E, Courtois P. EFFECT OF MICROFLORA ON THE IN VITRO ALTERATION OF HYDROCORTISONE 17-BUTYRATE ON HEALTHY OR PSORIATIC SKIN:
HPLC DETERMINATION. J Pharm Clin 1994;13(1):50-5.

An HPLC method was used to determine the effect of microflora on the in vitro alteration of hydrocortisone butyrate (hydrocortisone 17-butyrate; Locoid) on healthy and on psoriatic skin. The skin induced transformation of hydrocortisone butyrate into hydrocortisone at 37DGC, which was probably due to biological alteration from bacteria associated with skin microflora. In comparison with healthy skin, the increase in the number of bacteria such as Klebsiella and Streptococcus A has been observed with psoriatic skin. Therefore, it was concluded that a skin damaged by psoriasis may alter the hydrocortisone butyrate faster and in a larger amount than healthy skin.

173
Conti B, Puglisi G, Ventura CA, Giunchedi P, Cutuli V, et al. TOLMETIN POLY-D,L-LACTIDE MICROSPHERES: IN VITRO/IN VIVO EVALUATION. STP Pharma Sci 1994;4(4):269-74.

Poly-D,L-lactide microspheres containing tolmetin were prepared by

emulsification solvent evaporation or by spray drying and the microspheres were evaluated in vitro and in rats. Spherical shaped particles with a size of 3.5-7.5 mum and drug content of 4.5-9.5% were obtained. The dissolution of microspheres was dependent on the method of preparation, with drug release being slower from spray-dried microspheres. Biological evaluation showed the suitability of a microparticulate syste for chronic administration of the drug. The anti-inflammatory effect of tolmetin-loaded microspheres in rat models of acute and chronic inflammation was comparable to that of free drug, but significantly sustained. It was concluded that poly-D,L-lactide microspheres can be used for the sustained release of tolmetin.

174
Kobayashi D, Matsuzawa T, Sugibayashi K, Morimoto Y, Kimura M. ANALYSIS OF THE COMBINED EFFECT OF l-MENTHOL AND ETHANOL AS SKIN PERMEATION ENHANCERS
BASED ON A TWO-LAYER SKIN MODEL. Pharm Res 1994;11(Jan):96-103.

The effects of l-menthol ((-)-menthol) combined with ethyl alcohol (ethanol) on the percutaneous absorption of model drugs were studied using 2 equations

and a 2-layer in vitro skin model. A nonlinear least-squares method was employed to determine 6 coefficients using the 2 equations and experimentally obtained permeability coefficient through full-thickness skin and full-thickness skin/vehicle concentration ratio. Adding menthol to water and 40% ethyl alcohol increased the diffusion coefficient of drugs in lipid and pore pathways of stratum corneum. Adding ethyl alcohol to water and 5% menthol increased drug solubility in the vehicle, decreased skin polarity, and increased the role of the pore pathway to whole-skin permeation. It was concluded that the effects of l-menthol and ethyl alcohol on percutaneous drug absorption in vitro are synergistic.

GENOTOXICITY/MUTAGENESIS

175

Ronis MJ, Badger TM. TOXIC INTERACTIONS BETWEEN FUNGICIDES THAT INHIBIT ERGOSTEROL BIOSYNTHESIS AND PHYSPHOROTHIOATE INSECTICIDES IN THE MALE RAT AND BOBWHITE QUAIL (COLINUS VIRGINIANUS). Toxicol Appl Pharmacol 1995;130(2):221-8.

The potential for toxic interactions between ergosterol biosynthesis-inhibiting fungicides (EBIFs), used in U.S. agriculture or clinically, and phosphorothioate insecticides was assessed in adult male rats and adult male bobwhite quail (Colinus virginianus) by measuring inhibition of plasma butyryl cholinesterase (BChE) following fungicide and insecticide treatment. Male Sprague-Dawley rats (300 g) were administered corn oil or the following EBIFs: propiconazole (400 mg/kg/day), vinclozolin (400 mg/kg/day), clotrimazole (100 mg/kg/day), or ketoconazole (100 mg/kg/day) for 3 days by oral gavage. Forty-eight hours following the final dose, a single bolus of parathion (0.4 mg/kg in corn oil) or malathion (150 mg/kg in corn oil) or corn oil alone was given po. The rats were terminated 12 hr following parathion or 4 hr following malathion dosing. Significant ($p < 0.05$) inhibition of BChE was observed with parathion and malathion only following clotrimazole treatment. In contrast, when a similar experiment was performed in bobwhite quail dosed with 12 mg/kg malathion following EBIF treatment, significant BChE inhibition was observed following treatment with vinclozolin or ketoconazole, but not with propiconazole or clotrimazole. Induction of cytochrome P450 in rat and quail liver by EBIFs was accompanied by enhanced oxidative desulfuration of malathion, parathion, and diazinon to toxic oxon products. Increased detoxication via oxidative dearylation/esterolytic cleavage also occurred. However, while enhanced acute in vivo insecticide toxicity was observed in both species with a number of EBIF-phosphorothioate combinations, EBIF-induced oxidative activation of phosphorothioates by liver microsomes in vitro was not a good predictor of this effect.

176

Uuskula M, Jarventaus H, Hirvonen A, Sorsa M, Norppa H. INFLUENCE OF GSTM1 GENOTYPE ON SISTER CHROMATID EXCHANGE INDUCTION BY STYRENE-7,8-OXIDE AND 1,2-EPOXY-3-BUTENE IN CULTURED HUMAN LYMPHOCYTES. Carcinogenesis 1995;16(4):947-50.

Glutathione S-transferase M1 (GSTM1), catalyzing the conjugation of various reactive molecules with glutathione (GSH), shows genetic polymorphism in humans. Almost half of all Caucasians lack the GSTM1 gene, being theoretically at a higher risk from the toxic effects of substrates for GSTM1. The purpose of the present study was to investigate whether the GSTM1 genotype of lymphocyte donors influences the in vitro induction of sister chromatid exchanges (SCEs) by styrene-7,8-oxide (SO) and 1,2-epoxy-3-butene (MEB), the epoxide metabolites of styrene and butadiene respectively and potential substrates for GSTM1. SCEs induced after a 48 h treatment (started 24 h after culture initiation) by two different concentrations of SO (50 and 150 muM) and MEB (50 and 250 muM) were analyzed in cultured (72 h) lymphocytes of six GSTM1 null (gene deleted) and six GSTM1-positive (gene present) donors. Both SO and MEB were found to clearly increase SCEs. The GSTM1 genotype had no influence on SCE induction by SO. In contrast, MEB produced a higher level of SCEs among the GSTM1 null than GSTM1-positive samples. At 250 muM MEB, the GSTM1 null donors showed 31 % more induced SCEs (on average seven more SCEs per cell) than the GSTM1-positive donors (P = 0.02, acetone treatment as the reference). Furthermore, the GSTM1 null genotype was associated with a slight decrease in mitotic index and replication index, regardless of the treatment. The results suggest that GSTM1-mediated GSH conjugation is an important detoxification pathway for MEB, but not for SO, in cultured human lymphocytes.

177
Zeisig M, Moller L. 32P-HPLC SUITABLE FOR CHARACTERIZATION OF DNA ADDUCTS FORMED IN VITRO BY POLYCYCLIC AROMATIC HYDROCARBONS AND DERIVATIVES.
Carcinogenesis 1995;16(1):1-9.

Analysis of DNA adducts demands both high sensitivity and good resolution. A high-performance liquid chromatography method for 32P-postlabeled DNA adducts (32P-HPLC) was used to investigate DNA adduct formation from 38 polycyclic hydrocarbons and biphenyls in vitro. The 32P-HPLC method proved to be useful for separation, detection and characterization of DNA adducts from most of the substances. The in vitro method used to form the DNA adducts, with calf thymus DNA, nucleotide 3'phosphates and metabolic activation through S-9 liver homogenate, gave poor quantitative reproducibility. However, the results showed that the 32P-HPLC method was suitable for characterizing DNA adducts from many substances. From 35 of the tested substances 365 DNA and nucleotide 3'-phospate adducts were detected and characterized concerning retention times. Of the adducts, 171 were detected in DNA and 39 of them from five substances were characterized concerning target nucleotides. The retention time library built can be used in future analyses of DNA with complex patterns of DNA adducts.

178
Naji-Ali F, Hasspieler BM, Haffner D, Adeli K. HUMAN BIOASSAYS TO ASSESS ENVIRONMENTAL GENOTOXICITY: DEVELOPMENT OF A DNA REPAIR ASSAY IN HepG2 CELLS.
Clin Biochem 1994;27(6):441-8.

A direct assessment of the effects of environmental chemicals on human health has been hampered by the lack of suitable experimental systems. We have

recently employed a human liver cell line (HepG2) to assess the biological effects of pollutants at both cellular and DNA levels. A Neutral Red dye uptake assay was used to assess potential cytotoxic effects of xenobiotics. DNA damage was quantified using an unscheduled DNA synthesis assay that measures repair that is induced following exposure to genotoxic compounds. HepG2 cells responded to the known mutagens, 4-nitroquinoline N-oxide and methylmethane sulfonate, both in the Neutral Red assay for cytotoxicity and two DNA repair assays for genotoxicity (monitored autoradiographically or by liquid scintillation counting). The HepG2 DNA repair and cytotoxicity assays also responded to an extract (containing polycyclic aromatic hydrocarbons) of sediment obtained from a polluted site in the Great Lakes. Results indicate that this system can be deployed further to assess potential cyto- and genotoxicity of pollutants. The development of human cell culture assays is a critical step towards a full assessment of the risk that such pollutants pose to human health.

179
Mackay JM, Fox V, Griffiths K, Fox DA, Howard CA, Coutts C, Wyatt I, Styles JA. TRICHLOROACETIC ACID: INVESTIGATION INTO THE MECHANISM OF CHROMOSOMAL
DAMAGE IN THE IN VITRO HUMAN LYMPHOCYTE CYTOGENETIC ASSAY AND THE MOUSE BONE
MARROW MICRONUCLEUS TEST. Carcinogenesis 1995;16(5):1127-33.

Trichloroacetic acid (TCA) was tested for its ability to induce chromosomal damage in cultured human peripheral blood lymphocytes and in bone marrow cells of male and female C57BL/6JfBL10/Alpk mice. Two in vitro cytogenetic assays were conducted with TCA. In the first TCA, as free acid, was added to whole blood cultures at final concentrations of 500, 2000 and 3500 micrograms/ml in the presence and absence of an auxiliary metabolic activation system (rat liver S9-mix). Statistically significant increases in the percentage of aberrant cells compared with solvent control values were observed in cultures treated with TCA at 2000 and 5000 mu/ml. Investigation into the effects of TCA on the pH of the culture medium revealed significant reductions in pH at both these TCA concentrations. Neutralized TCA was then tested at concentrations of 500, 2,000 and 5000 micrograms/ml, also in the presence and absence of S9-mix. No statistically or biologically significant increases in the percentage of aberrant cells were observed in any of these cultures. In the mouse micronucleus test, neutralized TCA was administered in two equal intraperitoneal doses 24 h apart to C57BL/6JfBL10/Alpk mice (337, 675 and 1080 mg/kg in males; 405, 810 and 1300mg/kg in females). These dose levels represent 25%, 50% and 80% of the median lethal dose (MLD) in this strain of mouse. Bone marrow samples were taken 6 and 24 h after the second dose and the chromosomal damage assessed by analysis of the bone marrow for micronuclei. No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes compared with the solvent control dosed animals were observed in either sex at the 6 h sampling time or in the females at the 24 h sampling time. A small but statistically significant increase in micronucleated polychromatic erythrocytes was observed in male mice 24 h after a dose of 675 mg/kg (50% MLD). Since no increases were noted at the 25 or 80% MLD, and the levels recorded are within the range of the

concurrent solvent control values, the small increase observed in the males at the 50% LD is considered not to be biologically significant. Flow cytometric studies on suspensions of isolated liver cell nuclei revealed that changes in FITC binding (indicating altered chromatin conformation) were induced by pH changes alone and were not caused by neutralized TCA.(ABSTRACT TRUNCATED AT 400 WORDS)

180
Burgeot T, His E, Galgani F. THE MICRONUCLEUS ASSAY IN CRASSOSTREA GIGAS FOR THE DETECTION OF SEAWATER GENOTOXICITY. Mutat Res 1995;342(3-4):125-40.

The micronucleus (MN) test was performed in vivo and in vitro on the oyster Crassostrea gigas to evaluate the genotoxic effect of the marine environment. In vitro tests were carried out on adult and young (spat) specimens exposed to benzo[a]pyrene (BaP: 0.5, 5, 500 and 1000 micrograms.l-1) and an effluent (5, 50, 75 and 100%) of Seine Bay, one of the most highly contaminated sites in France. MN frequency observed after 48 h exposure to the two pollutants was much greater in adults than spats. A preliminary test of the genotoxic effect of BaP (0.05, 0.5, 1 and 500 micrograms.l-1), cupric sulfate (10, 25, 50 and 100 micrograms.l-1) and a paper mill effluent (1, 3, 10 and 30 mg.l-1) was performed in C. gigas heart cells cultured for 6 days. Comparison of the MN assay with the C. gigas larva test showed the clastogenic action of BaP and the toxic effect of cupric sulfate on culture cells as well as the slighter toxic effect of paper mill effluent on spats. An in vivo study was conducted in an oyster-farming area contaminated by cadmium and copper. MN frequency was not very sensitive to a pollution gradient but showed high interindividual variability. The absence of precise criteria for MN identification in mollusks and the identification of highly basophilic spherical inclusions in the cytoplasm of gill tissue hemocytes in oysters during viral infection are handicap for application of the micronuclei assay in the marine environment. Another limitation of the assay is the particularly onerous requirement for manual observation. Optimization of the assay by automated analysis is necessary but can only be achieved if cytologic preparations are of good quality.

181
Gollapudi BB, Linscombe VA, Wilmer JW. CLASTOGENICITY OF ISOAMYLENE OXIDE TO RAT LYMPHOCYTES IN CULTURE. Mutat Res 1995;347(1):9-12.

The mutagenic activity of the aliphatic epoxide isoamylene oxide (2-methyl-2,3-epoxybutane) is not readily detectable in the standard Ames test. In this study, the clastogenic potential of isoamylene oxide was evaluated using an in vitro mammalian cell culture system. Approximately 48 h after establishing primary cultures of rat lymphocyte cultures, the cells were treated for 4 h with various concentrations of isoamylene oxide (50, 166.7, 500,, 1666.7, and 5000 micrograms/ml in the initial assay and 500, 1000, 2000, 3000, 4000, and 5000 micrograms/ml in the confirmatory assay). The cultures were harvested 24 h after termination of the treatment. Based upon the mitotic indices, cultures treated with the three highest concentrations in both the initial and confirmatory assays were evaluated to estimate the chromosomal aberration frequencies. Isoamylene oxide demonstrated a strong clastogenic

activity in this assay: up to 29% aberrant cells (without gaps) were observed at the highest concentration analyzed. The presence of an external metabolic activation system (S9) did not seem to influence the magnitude of the response at the dose levels analyzed.

182
Foellmann W, Hillebrand IE, Creppy EE, Bolt HM. SISTER CHROMATID EXCHANGE FREQUENCY IN CULTURED ISOLATED PORCINE URINARY BLADDER EPITHELIAL CELLS (PUBEC) TREATED WITH OCHRATOXIN A AND ALPHA. Arch Toxicol 1995;69(4):280-6.

The mycotoxin ochratoxin A (OTA) and its metabolite ochratoxin alpha (OT-alpha) were investigated, to examine their potency to induce sister chromatid exchanges (SCE) in cultured porcine urinary bladder epithelial cells (PUBEC) (primary culture). Serum-free cultured PUBEC were incubated for 5 h with either OTA or OT-alpha, respectively, and subsequently cultured in the presence of 5-bromo-2-deoxyuridine (BrdU). After two cell cycles, mitosis was inhibited by the colchicine derivative Colcemid, cells were fixed and chromosomes were prepared for SCE analysis. For OTA, a dose-dependent increase in SCE frequency was measured in concentrations between 100 pM and 100 nM OTA. At 100 nM OTA, SCE frequency increased by about 41%, compared to the base SCE level (7.27 SCEs per chromosome set, solvent control). Higher concentrations of OTA were cytotoxic. The metabolite OT-alpha also increased SCE frequency, but at higher concentrations. At a concentration of 10 muM OT-alpha, an increase of about 55% was detected. OT-alpha showed no cytotoxic effect. These results indicate that OTA is genotoxic in this in vitro system, which represents the urinary bladder epithelium, a target organ of OTA in vivo. It could also be shown that OT-alpha, which is said to be non-toxic, is genotoxic in this assay at higher concentrations.

183
Mao B, Xu J, Li B, Margulis LA, Smirnov S, Ya NQ, Courtney SH, Geacintov NE. SYNTHESIS AND CHARACTERIZATION OF COVALENT ADDUCTS DERIVED FROM THE BINDING OF
BENZO(A)PYRENE DIOL EPOXIDE TO A-GGG- SEQUENCE IN A DEOXYOLIGONUCLEOTIDE. Carcinogenesis 1995;16(2):357-65.

Direct synthesis and purification procedures are described for the preparation of adducts derived from the covalent binding of 7R,8S-dihydroxy-9S,10R-epoxy-7,8,9,10-tetrahydro-benzo(a)pyrene ((+)-anti-BPDE or (+)-BPDE 2) to each of the three guanine residues (trans-N2-dG lesions) in the oligodeoxyribonucleotide d(CTATG1G2G3TATC). The positions of the modified Gs are defined by Maxam-Gilbert sequencing techniques. Six different oligonucleotides with one or two precisely positioned (+)-anti-BPDE residues are identified. The absorbance, circular dichroism and fluorescence characteristics are changed upon formation of duplexes with the complementary strands d(GATACCCATAG). In the doubly-modified oligonucleotides, a broad, excimer-like long wavelength fluorescence emission band is observed with a maximum near 455 nm only if the two (+)-anti-BPDE-modified Gs are adjacent to one another. The covalently attached (+)-anti-BPDE residues decrease the thermodynamic stabilities of the duplexes; their melting points are markedly dependent on the position of the lesions, being highest with the (+)-anti-

BPDE residue at G1 (Tm = 40~C, only 2~C lower than in the case of the unmodified oligonucleotide) and lowest when it is situated at G3 (Tm = 29~C). The implications of these and other physical characteristics are discussed. The facile synthesis of these or similar site-specific and stereochemically defined (+)-trans-anti-BPDE-N2-dG lesions in runs of contiguous guanines in oligodeoxyribo-nucleotides of specified base sequence should be useful for the design of site-directed mutagenesis studies in vitro and in vivo.

184
Morrison V, Ashby J. HIGH RESOLUTION RODENT BONE MARROW MICRONUCLEUS ASSAYS OF
1,2-DIMETHYLHYDRAZINE: IMPLICATION OF SYSTEMIC TOXICITY AND INDIVIDUAL RESPONDERS. Mutagenesis 1995; 10(2):129-35.

1,2-Dimethylhydrazine (DMH) is confirmed as active in male CBA mouse bone marrow micronucleus (MN) assays conducted at non-toxic dose-levels between 25 and 45 mg/kg. Previous reports of the activity of DMH in rodent bone marrow MN assays are reviewed. By the design of the present experiments it has been possible to confirm the advice offered by the United Kingdom Environmental Mutagen Society (UKEMS) that once 2000 polychromatic erythrocytes have been assessed per animal, the use of larger animal group sizes becomes the critical means to increase assay sensitivity. Also, the use of historical control data is shown to enhance assay sensitivity at low dose-levels by enabling recognition of individual animals responding to the test chemical. These outliers are seen before the group mean MPE value has been increased enough to attain group statistical significance. It is concluded that careful consideration of test data, within the context of concurrent and historical control data, is sufficient to discern activity in the assay.

185
Bolcsfoldi G. THE DNA ALKALINE UNWINDING GENOTOXICITY TEST. Methods Mol Biol 1995;43:257-66.

186
Zhu AX, Zhao Y, Moller DE, Flier JS. CLONING AND CHARACTERIZATION OF p97MAPK, A NOVEL HUMAN HOMOLOG OF RAT ERK-3. Mol Cell Biol 1994; 14(12):8208-11.

Mitogen-activated protein kinases, or extracellular signal-regulated kinases (ERKs), are serine/threonine protein kinases that are activated in response to a wide variety of extracellular stimuli and are encoded by a multi-gene family. Little is known about the function of the ERK-3 subfamily. To explore the mol. diversity of the ERK-3 subfamily, the authors isolated a novel human cDNA, designated Hu-ERK-3, from a fetal skeletal muscle library. Anal. of the complete 3,920-bp nucleotide sequence revealed that this clone encodes a predicted protein of 720 amino acids. In vitro transcription-translation generates a 97-kDa protein referred to as p97MAPK. Of all of the sequences compared, p97MARK is the most homologous to rat ERK-3. Interestingly, although p97MAPK is highly (98%) homologous to ERK-3 at the amino acid level within the N-terminal two-thirds of the coding region, it diverges at the carboxyl terminus as a result of a unique extension of 178 amino acids. Although expression of p97MAPK was detected in all of the

tissues tested by Northern (RNA) anal., the most abundant expression was seen in skeletal muscle. An antibody in an immune complex protein kinase assay, the authors have shown that treatment of human fibroblasts with serum or phorbol esters activates a myelin basic protein and histone H1 kinase activity in immunoppts. P97MAPK appears to be the human homolog of rat ERK-3, and a member of this family is an active protein kinase.

187
Hasspieler BM, Ali FN, Alipour M, Haffner GD, Adeli K. HUMAN BIOASSAYS TO ASSESS ENVIRONMENTAL GENOTOXICITY: DEVELOPMENT OF A DNA BREAK BIOASSAY IN
HepG2 CELLS. Clin Biochem 1995;28(2):113-6.

An in vitro assay was developed for the quantification of genotoxicity, monitored as DNA single-strand breaks (SSB), in the HepG2 human hepatoma cell line. This assay procedure, which is based upon alk. unwinding and hydroxylapatite DNA chromatog., is both rapid and simple to perform. HepG2 cells responded to the std. mutagen, 4-nitroquinoline N-oxide, demonstrating SSB formation at concns. >0.1 mumol/L. Phenanthrene-9,10-quinone, a component of diesel exhaust, mediated SSB formation at concns. >250 nmol/L. Finally, an ext. of contaminated sediment from the Great Lakes Basin mediated SSB formation in a dose-dependent manner. These results illustrate the utility of this human genotoxicity assay for future use in screening of environmental pollutants.

188
Gu Z. [IN VITRO MICRONUCLEUS ASSAY IN BALB/C-3T3 CELLS]. Gongye Weisheng Yu Zhiyebing 1995; 20(4):204-6. (Chi)

The genotoxicity of benzo(a)pyrene, cyclophosphamide, 2-aminoanthracene, 2-nitrofluorene, nitrosated coal-dust exts., and cigaret-smoke condensate were tested with the in vitro micronucleus assay using an established BALB/c-3T3 mammalian cell line. The results showed that all chems. and complex mixts. studied induced micronuclei in cells tested. These results indicate that BALB/c-3T3 cells are capable of activating certain promutagens and procarcinogens. It seems, therefore, that the micronucleus assay in BALB/c-3T3 cells without an exogenous activation system may be useful as an in vitro assay for detecting genotoxicity.

189
Gollapudi BB, Mendrala AL, Linscombe VA. EVALUATION OF THE GENETIC TOXICITY OF THE ORGANOPHOSPHATE INSECTICIDE CHLORPYRIFOS. Mutat Res 1995;342(1-2):25-36.

The genetic toxicity of chlorpyrifos (O,O,-diethyl-O-(3,5,6-trichloro-2-pyridinyl)phosphorothioate, C.A.S. Number: 2921-88-2)), an organophosphate insecticide, was examined by employing several end points such as gene mutations in bacteria (Ames test) and mammalian cell cultures (CHO/HGPRT assay), cytogenetic abnormalities in mammalian cells both in vitro (rat lymphocyte chromosomal aberration test, RLCAT) and in vivo (mouse bone marrow micronucleus test) and induction of DNA damage and repair in rat hepatocytes in vitro. There was no indication of genotoxic activity for

chlorpyrifos in any of these assays. These results are consistent with the reported lack of carcinogenic potential for chlorpyrifos in both mice and rats.

190
Bond JA, Recio L, Andjelkovish D. EPIDEMIOLOGICAL AND MECHANISTIC DATA SUGGEST THAT 1,3-BUTADIENE WILL NOT BE CARCINOGENIC TO HUMANS AT EXPOSURES LIKELY TO
BE ENCOUNTERED IN THE ENVIRONMENT OR WORKPLACE. Carcinogenesis 1995;16(2):165-71.

1,3-Butadiene (BD) is a carcinogen in both rats and mice with mice being substantially more sensitive than rats. It is not known if BD poses a carcinogenic risk for humans. Findings from exposure assessment studies indicate that potential industrial exposure to BD in monomer, polymer, and end-user industries is typically <2 p.p.m. Epidemiologic studies of persons occupationally exposed to BD are inconclusive. In vitro metabolism of BD in rats, mice and human tissues indicate that there are significant quantitative species differences in the metabolic activation of BD to butadiene monoepoxide (BMO) and butadiene diepoxide (BDE) and the detoxication of BMO. Activation/detoxication ratios calculated using in vitro kinetic constants reveal that ratios in mice were 12-fold greater than rats and humans. In rats and mice exposed to BD, concentrations of BMO in blood and tissues of mice were up to 14-fold higher than in rats and BDE was only detected in mice thereby providing a strong argument for why mice are highly sensitive to BD carcinogenicity. The fact that human tissues do not appear to metabolize BMO to BDE to any significant extent suggest that humans may not be sensitive to BD carcinogenicity. In mice, BDE is a more potent carcinogen than BMO. BDE is mutagenic in vitro at the hprt locus in human TK6 lymphoblasts at concentrations that were 100-fold less than the concentration of BMO required to yield a similar mutation frequency. Importantly, the concentrations of BDE that were genotoxic in vitro are nearly identical to the concentrations of BDE measured in blood and tissues of mice exposed to BD by inhalation. BD is genotoxic in mice, but not rats, following inhalation exposure and this is paralleled by species differences in observed tumor susceptibility. BD is not genotoxic in occupationally-exposed workers. The genetic basis for BD carcinogenicity appears to be primarily through induction of point mutations and deletion events mediated via the potent genotoxic metabolite, BDE. The genotoxic endpoints induced by BDE (e.g., deletion and point mutations) rather than BMO (e.g., point mutations) likely represent the underlying mechanism responsible for the striking species differences observed in the genotoxicity and carcinogenicity of BD in mice versus rats. In summary, the preponderance of evidence which includes both epidemiological and mechanistic data in mice, rats, and humans strongly suggests that BD will not be carcinogenic to humans at occupational or environmental exposures. Any cancer risk assessment for BD should use in vitro human tissue metabolic data and in vitro and in vivo rat data for estimation of human cancer risks.

191
Yamasaki H. NON-GENOTOXIC MECHANISMS OF CARCINOGENESIS: STUDIES OF CELL

TRANSFORMATION AND GAP JUNCTIONAL INTERCELLULAR COMMUNICATION. Toxicol Lett
1995;77(1-3):55-61.

It is widely accepted that a series of genetic changes accumulate during carcinogenesis. In addition, it is likely that various non-genotoxic mechanisms also operate at different stages of carcinogenesis. It is even possible that non-genotoxic mechanisms indirectly generate genetic changes, e.g., through induction of cell proliferation, active oxygen species or cytosine methylation. This may partially explain why many carcinogens are devoid of activity when tested in the usual genetic toxicology assays. In vitro cell transformation mimics certain stages of in vivo carcinogenesis. It has therefore been proposed that both genotoxic and non-genotoxic aspects of carcinogenesis can be studied in cell transformation systems, with tumor formation by transformed cells in syngenic animals or nude mice as the endpoint. Many genotoxic as well as non-genotoxic carcinogens induce transformation of Syrian hamster embryo, murine Balb/c 3T3 and murine C3H10T1/2 cells; interaction of genotoxic and non-genotoxic mechanisms can be clearly seen in 2-stage cell transformation studies in which a genotoxic initiating agent and a non-genotoxic promoting agent act synergistically to induce transformation of rodent cells. Aberrant control of gap junctional intercellular communication (GJIC) in cell transformation and carcinogenesis is well documented. Possible genotoxic as well as non-genotoxic mechanisms involved in abnormal gap junction communication control in multistage carcinogenesis are discussed.

192
Shaw G, Connell D, Barron W. THE USE OF IN VITRO DNA ADDUCT FORMATION TO ESTIMATE THE GENOTOXICITY OF RESIDUES AT CONTAMINATED SITES. Chemosphere 1995;30(10):1957-68.

Genotoxic carcinogens such as polycyclic aromatic hydrocarbons (PAHs) covalently bind to the bases in DNA to form adducts. The formation of DNA adducts is significant with respect to chemical carcinogenesis. Many contaminated sites contain quantities of carcinogens such as PAHs, and the evaluation of the genotoxicity of these soils has important implications for human risk assessment. DNA adducts can be formed using an in vitro system incorporating extracts from contaminated soils. The 32P-postlabelling assay is a sensitive technique for the detection of DNA adducts from complex mixtures of environmental carcinogens. These techniques have been used to form and detect DNA adducts using soils from a number of coal gasworks sites. The results show that the extent of adduct formation depends partially on the petroleum hydrocarbon content of samples, but also on other undetermined factors related to composition. While environmental weathering has been shown to effect the PAH composition of samples, this is not an important factor in controlling the genotoxicity of samples as estimated by DNA adduct formation.

193
Santambien P, Sdiqui S, Hubert E, Girot P, Roche AC, Monsigny M, Boschetti E. IN VITRO TOXICITY ASSAYS FOR DYE LIGANDS USED IN AFFINITY CHROMATOGRAPHY. J Chromatogr B Biomed Appl 1995;664(1):241-6.

Some reactive textile dyes have been used for years as biomimetic ligands in protein purification. There has been reluctance, however, to use these dyes on a large scale for therapeutically applicable proteins for fear of possible dye leakage and consequent contamination. Therefore, toxicological data are necessary to quantify the level of this hazard. This study deals with a series of in vitro toxicity investigations with eukaryotic cells (growth, polyploidy, etc.) and with prokaryotic cells (Escherichia coli) for genotoxic studies. Both approaches demonstrated a lack of or slight toxicity for Reactive Blue 2 and Reactive Red 120 and their derivatives over the range 10-62.5 micrograms/ml in several assays.

194
Parry EM, Henderson L, Mackay JM. PROCEDURES FOR THE DETECTION OF CHEMICALLY INDUCED ANEUPLOIDY: RECOMMENDIATIONS OF A UK ENVIRONMENTAL MUTAGEN SOCIETY
WORKING GROUP. Mutagenesis 1995;10(1):1-14.

The development of assays to detect numerical chromosome aberrations has not kept pace with that for assays used to detect other genotoxicity endpoints such as gene mutations and structural chromosome aberrations, even though the importance of aneuploidy in relation to heritable defects in germ cells and to carcinogenesis in somatic cells is acknowledged. Regulatory bodies at present have no formal requirements concerning aneuploidy detection and decisions are made on a case-by-case basis. The aim of this review is to indicate which assays are available for the detection of chemically induced aneuploidy and what aspects should be taken into account when testing for chemically induced aneuploidy using in vitro, in vivo somatic and in vivo germ cell assays without dictating exact protocols. Our recommendations concentrate on systems that, to date, have been most extensively used and we indicate where future developments may lie. It is important that the currently available and future tests for chemically induced aneuploidy should be adequately validated before being implemented into screening strategies or regulatory guidelines. This requirement has not yet been met and is confounded by the lack of a well defined reference database of animal and human chemical aneugens.

195
Tateno H, Kamiguchi Y. APPLICATION OF CRYOPRESERVED GOLDEN HAMSTER OOCYTES TO
IN VITRO GENOTOXICITY ASSAYS FOR HUMAN SPERM CHROMOSOMES. Environ Mol Mutagen
1995;25(3):263-5.

196
Smith ML, Chen IT, Zhan Q, O'Connor PM, Fornace AJ Jr. INVOLVEMENT OF THE p53 TUMOR SUPPRESSOR IN REPAIR OF U.V.-TYPE DNA DAMAGE. Oncogene 1995;10(6):1053-9.

The tumor suppressor p53 plays a central role in the cellular responses to genotoxic stress. Besides its well known role in activation of the G1 checkpoint after exposure to agents like ionizing radiation and its role in

apoptosis, the possibility exists that p53 may have additional roles, such as in DNA repair. For example, p53, is known to bind to single strand DNA such as would occur during repair events, and the proteins encoded by two p53-regulated genes have previously been found to bind to at least one protein involved in DNA damage processing including nucleotide excision repair (NER). NER is an important and versatile DNA repair mechanism, which is the major pathway for repair of u.v.-type lesions and damage by a variety of important carcinogens and mutagens. If components of the p53 pathway are involved in NER, then disruption of p53 function by mutations or expression of certain viral proteins could have important implications in carcinogenesis and cancer treatment. In the present study we show that disruption of normal p53 function in human colon carcinoma RKO cells with either the human papillomavirus E6 oncoprotein or a dominant-negative mutant p53 transgene results in reduced repair of u.v.-induced DNA damage. The E6 and mutant p53-containing cell lines demonstrated reduced repair of u.v.-induced DNA lesions in host cell reactivation experiments with reporter plasmids, and reduced repair in in vitro DNA repair assays. With this in vitro assay, extracts from the E6- and mutant p53-containing lines also showed loss of induced repair following cellular u.v.-irradiation. The reduced DNA repair activity of the transfected cell lines also correlated with reduced clonogenic survival following u.v.-irradiation. These results indicate that p53 and/or p53-regulated gene products function in the NER pathway and that this process is inducible by DNA damage.

197
Sdiqui N, Santambien P, Roche AC, Hebert E, Girot P, Cochet S, Boschetti E, Monsigny M, Bertrand O. TOXICITY STUDIES ON NATIVE PROCION RED HE-3B AND RELEASED DYE FROM AFFINITY MATERIAL EXPOSED TO DEGRADATIVE CHEMICAL CONDITIONS. J Biochem Biophys Methods 1994;29(3-4):269-82.

Leached ligands from chromatographic packing material submitted to drastic regeneration conditions can contaminate pure biological preparations. These contaminants could have adverse effects from a toxicology point of view that are very poorly documented in liquid chromatography for protein separation. Investigations on toxicity level have been made on released material from immobilized Procion Red HE-3B, after formal identification of the nature of the leached chemical material. Toxicity investigations in vitro involved a number of tests on living cells (eucaryotic and procaryotic) covering different aspects. Behaviour of cells in regular cultures, polyplo:idia induction, genotoxicity as well as mechanisms of endocytosis have been studied. Results showed no toxic effects within the range of concentration of dye and dye derivatives studied. Genotoxicity studies in particular did not show any toxic effect over a range of concentration much higher than the regular level of dye leakage from the sorbent.

198
Marshall R. MEASUREMENT OF CHROMOSOME ABERRATIONS IN VITRO USING HUMAN PERIPHERAL BLOOD LYMPHOCYTES. Methods Mol Biol 1995, 43:287-96.

199
Shaw G. AN EVALUATION OF MOLECULAR BIOLOGICAL AND CYTOGENETIC TESTING FOR

DETERMINATION OF HUMAN RISK FROM EXPOSURE TO CHEMICALS PRESENT IN CONTAMINATED
SITES. J Environ Sci Health C 1995;13(1):75-103.

200
Mark H FL, Naram R, Singer JT, Rice RW, Bastan B, Beauregard LJ, Lamarche PH.
CYTOTOXICITY AND GENOTOXICITY OF WOOD DRYING CONDENSATE FROM SOUTHERN YELLOW
PINE: AN IN VITRO STUDY. Mutat Res 1995;342
(3-4):191-6.

We tested condensates from Southern Yellow Pine for potential cytotoxicity and genotoxicity in CHO-WBL and human peripheral blood lymphocytes (PBL) in the absence of S-9 activation. Cytotoxicity was evaluated by the Trypan blue exclusion assay, mitotic index (MI) and proliferative rate index (PRI). Genotoxicity was measured by the chromosome aberration (CA) assay and sister chromatid exchange (SCE) analysis. Both cytotoxic and genotoxic effects were observed. Laboratory-generated Southern Yellow Pine condensate reduced the viability of CHO-WBL cells. The number of viable cells was roughly inversely proportional to dosage over a range of 100% to 31% in treated groups, in both experiments, as compared to 2.60%) in the control. The MI data in both CHO cells and PBL also showed an inverse correlation. The highest scorable dose limited by toxicity was determined to be 1 ml of Southern Yellow Pine condensate in 10 ml total of medium. Lastly, a dose response curve was observed in CHO cells, as well as in PBL, using the CA assay and also with the SCE analysis. The present findings corroborate the results from Ames testing and represent the only information currently available on the genotoxic potential of these chemicals.

201
Shi CY, Hew YC, Ong CN. INHIBITION OF AFLATOXIN B1-INDUCED CELL INJURY BY

SELENIUM: AN IN VITRO STUDY. Hum Exp Toxicol 1995;14(1):55-60.

Dietary selenium is an essential trace element in human nutrition. Selenium has been shown in animal studies to inhibit aflatoxin hepatocarcinogenesis. However, the cellular mechanism responsible for the inhibition has not been thoroughly studied. This study examines the effect of two selenium compounds, namely, sodium selenite and selenium-enriched yeast extract (SeY), on the cytotoxicity, DNA-binding and mutagenicity of aflatoxin B1 (AFB1) in cultured Chinese hamster ovary (CHO) cells. CHO cells, after treatment with 2 mug ml-1 selenite or 80 mug ml-1 SeY, exhibited increased resistance to AFB1-induced cell killing. At a concentration of 50 mug ml-1 AFB1, cell survival, measured by the clonogenicity assay, was increased by 21- and 10-fold in selenite- and SeY-treated cells, respectively. However, selenium treatment did not appear to affect AFB1-DNA binding. Similarly, no effect was observed on AFB1 mutagenicity, as determined by the hypoxanthineguanine phosphoribosyl transferase (HPRT) gene mutation assay. The results showed that selenium could effectively protect cells from AFB1 cytotoxicity in cultured cells but had no effect on AFB1-DNA adduct formation or mutagenesis. It is suggested that there are multiple pathways of AFB1 toxicity and that selenium can modulate

AFB1-induced cell killing independent of its genotoxicity.

202
Venkat JA, Shami S, Davis K, Nayak M, Plimmer JR, Pfeil R, Nair PP. RELATIVE GENOTOXIC ACTIVITIES OF PESTICIDES EVALUATED BY A MODIFIED SOS MICROPLATE ASSAY. Environ Mol Mutagen 1995;25(1):67-76.

The genotoxic activities of 47 pesticides were determined using a modified SOS microplate assay in which the induction of beta-galactosidase in E. coli PQ37 was used as a quantitative measure of genotoxic activity. The results were compared with those obtained with anethole, curcumin, and capsaicin, a few examples of naturally occurring compounds present in foods. The assays were conducted with pesticides dissolved either in a suitable solvent, such as 10% DMSO in physiological saline or dispersed in sodium taurocholate micelles, to simulate conditions in the small intestine from where these substances are normally absorbed from the diet. 4-Nitroquinoline oxide (4-NQO) served as the reference standard of a direct acting mutagen. In micellar form, 4-NQO and 25 of the 47 pesticides tested showed significantly higher genotoxic activities than when they were tested in an organic solvent. In micellar form the SOS inducing potency of 4-NQO was almost twice as high as in 10% DMSO in physiological saline. In taurocholate micelles, the five most active compounds had activities in the range of 1,234-3,765 units/ mumol and in the order of decreasing activities they were ranked as follows: malathion > dichlorvos > lindane > chlordane > endrin. They were significantly less active than 4-NQO (less than 40%). In micellar solution the naturally occurring compounds, anethole, curcumin, and capsaicin gave activities of 4,594, 928, and 809 units/mumol, respectively. These studies show that genotoxicity may depend upon the environment in which cells are exposed to these potential genotoxins. It appears that testing of the more hydrophobic compounds, both synthetic and naturally occurring, are needed.

203
Coates PJ, Save V, Ansari B, Hall PA. DEMONSTRATION OF DNA DAMAGE/REPAIR IN INDIVIDUAL CELLS USING IN SITU END LABELLING: ASSOCIATION OF p53 WITH SITES OF DNA DAMAGE. J Pathol 1995; 176(1):
19-26.

We describe the development and application of in situ end labelling (ISEL) to identity sites of damaged DNA in the nuclei of individual cells. In cell culture, exposure to a variety of genotoxic agents induced a dose and time-dependent increase in nuclear labelling. In addition, examination of histological sections of human skin exposed to solar-stimulated UV light showed ISEL in both keratinocytes and superficial dermal cells, with the same spatial and temporal distribution as that of a marker of DNA repair, PCNA (proliferating cell nuclear antigen). Using co-localization techniques and confocal microscopy, we found increased levels of p53 in many ISEL-positive cells in vitro, with a similar distribution of labelling in the nucleus. This observation provides further evidence for a direct role of p53 in the recognition of damaged DNA. Thus, ISEL should prove a convenient method for demonstrating genotoxic insult in individual cells and in histological material, and may have value in toxicological screening. This high-resolution

microscopy technique can also be used to compare the spatial distribution of various proteins implicated in the response to DNA damage with the sites of the lesion.

204
Benigni R. MOUSE BONE MARROW MICRONUCLEUS ASSAY: RELATIONSHIPS WITH IN VITRO
MUTAGENICITY AND RODENT CARCINOGENICITY. J Toxicol Environ Health 1995;45(3):337-47.

In this article, the relationship was studied between the in vivo mutagenicity assay of mouse bone marrow micronucleus (MIC), and four in vitro assays: Salmonella typhimurium, chromosomal aberrations in Chinese hamster ovary (CHO) cells, sister chromatid exchanges (SCE) in CHO cells, and mutation in mouse lymphoma L5178Y cells. A comparison with the rodent carcinogenicity data was also undertaken. The MIC data on 49 chemicals were generated by Shelby et al. (1993). The MIC assay system employed three daily exposures by intraperitoneal injection; bone marrow samples were obtained 24 h following the final exposure. A preliminary analysis indicated that the 49 chemicals selected by Shelby et al. (1993) are a representative subset of the National Toxicology Program database. This study showed that MIC has a number of particular characteristics that are not shared by other biological systems. MIC is basically different from rodent carcinogenicity, despite being an in vivo system. At the same time, it responds to the chemicals in a different way from that of the in vitro genotoxicity assays. These in vitro assays mainly differ from each other in their different sensitivities to the genotoxins: MIC gives just a few positives (limited sensitivity), but, at the same time, some of these positives are detected only by the most sensitive assays, like the mouse lymphoma or SCE assays. In terms of risk assessment, MIC does not complement Salmonella for predicting chemical carcinogenicity, and would be better used to verify if the in vitro positive chemicals are able to exert their genotoxic potential in vivo.

205
James NH, Ashby J, Roberts RA. ENHANCED HEPATOCYTE COLONY GROWTH IN SOFT AGAR
AFTER IN VIVO TREATMENT WITH A GENOTOXIC CARCINOGEN: A POTENTIAL ASSAY FOR
HEPATOCARCINOGENS? Cancer Lett 1995;93(1):121-8.

We have shown previously that approximately 1 in 10,000 primary hepatocytes isolated from untreated rats undergo clonal growth in soft agar in vitro in response to the synergistic action of nafenopin, a peroxisome proliferator (PP) and epidermal growth factor (EGF), a naturally occurring liver growth regulator. Here, we demonstrate that prior treatment of the animals with the genotoxic hepatocarcinogen diethylnitrosamine (DEN) caused a dose-dependent increase in soft agar colony numbers formed in vitro. These data suggest that the colony assay may offer a method of detecting in vitro hepatocytes transformed in vivo by DEN. It is known that rats treated with DEN develop enzyme altered foci prior to the development of tumours. The majority of these foci express high levels of gamma-glutamyl transpeptidase (GGT). However, foci

promoted by PPs do not show this increased enzyme activity. In the present study, the colonies we have generated in vitro mimicked this pattern since the majority (approximately 80%) of the spontaneous colonies expressed GGT whereas colonies promoted by the synergistic action of nafenopin and EGF were mainly (75%) GGT negative. The proportion of colonies positive for GGT were similar using either hepatocytes isolated from control or from DEN-initiated rats. Further studies are required to assess if the hepatocytes selected for clonal expansion by this EGF/nafenopin regime reflect the presumed pre-neoplastic cells induced by genotoxin in vivo and associated with an increased propensity to cancer.

206
Hill CM, Lunec J, Griffiths HR, Herbert KE. CHARACTERIZATION OF TUMOR NECROSIS FACTOR ALPHA RELEASE BY HUMAN GRANULOCYTES IN RESPONSE TO PROCAINAMIDE CHALLENGE. Biochem Pharmacol 1995; 49(12):1837-49.

The role of human granulocytes in the promotion of procainamide (PA) toxicity in vitro has been studied and one of the agents responsible for DNA strand scission and cell death in human target cells has been characterized. Crude peripheral blood mononuclear cells (cPBMNs) isolated by density centrifugation, and the lymphocyte cell lines--CCRF-HSB2 and WIL-2NS--were exposed to PA, and DNA strand breaks were quantified by fluorescent analysis of DNA unwinding. Therapeutic plasma concentrations of PA (0-50 microM) caused dose-dependent cytotoxicity, determined by dye exclusion, and strand breaks in cPBMNs incubated for 3 and 1.5 hr at 37 degrees, respectively. Using 50 microM PA a five-fold increase in DNA strand breaks was observed after 1.5 hr, with significant induction of strand breaks also being observed for 10 and 25 microM concentrations. Toxicity was much reduced in lymphocyte cell lines (maximal killing = 3.0% at 50 microM PA compared with 13.2% in cPBMNs). A similar decrease in toxicity was observed where N-acetyl procainamide (NAPA) was substituted for PA (less than 50% of strand breaks at all concentrations). Further investigations showed that the presence of a contaminating granulocyte population in the cPBMN fraction was responsible for the induction of PA toxicity. Incubation of a highly enriched granulocyte population with PA for 1 hr prior to exposure to purified peripheral blood mononuclear cells (pPBMNs) led to the complete restoration of the toxic effects. The resulting cyto- and genotoxicity were not significantly different to levels observed in cPBMNs. Significantly, incubation of granulocytes with NAPA did not induce toxicity in target pPBMNs. Ultrafiltration of granulocyte supernatants led to the identification of two toxic fractions of < 3000 and > 30,000 Da. Temporal studies showed that the toxicity associated with the < 3000 Da fraction appeared during the first 10-15 min incubation with PA whereas the > 30,000 Da fraction did not display significant toxicity until the 40-60 min period. Further assessment of the nature of these agents indicated that the 30,000 Da fraction was a protein. SDS-PAGE analysis showed an inducible 17,800 Da species appearing in granulocyte supernatants fter 40 min incubation with PA. Dot blot analysis indicated that tumour necrosis factor alpha (TNF alpha) was present in the > 30,000 Da fraction. Evidence that TNF alpha was the high-molecular weight species responsible for PA-induced toxicity was obtained from neutralization assays employing an anti-TNF alpha antibody.(ABSTRACT TRUNCATED AT 400 WORDS)

207
Caria H, Chaveca T, Laires A, Rueff J. GENOTOXICITY OF QUERCETIN IN THE MICRONUCLEUS ASSAY IN MOUSE BONE MARROW ERTYHROCYTES, HUMAN LYMPHOCYTES, V79
CELL LINE AND IDENTIFICATION OF KINETOCHORE-CONTAINING (CREST STAINING) MICRONUCLEI IN HUMAN LYMPHOCYTES. Mutat Res 1995;343(2-3):85-94.

Quercetin, a mutagenic flavonoid widely distributed in edible plants, was studied for the induction of micronuclei (MN). We have carried out the MN assay in bone marrow polychromatic erythrocytes in mice, in cytokinesis-blocked human lymphocytes and in cytokinesis-blocked V79 cells. MN assay in vitro was performed in the presence and in the absence of S9. To further extend the study, an antikinetochore antibody (CREST staining) was used to distinguish MN containing whole chromosomes (kinetochore positive) from those containing acentric fragments (kinetochore negative). When tested in vivo quercetin failed to induce micronuclei, a result which is in agreement with other published reports. When tested in vitro in V79 cells quercetin clearly induces micronuclei in the absence of S9 and also in the presence of S9 for the highest dose used. When tested in vitro in human lymphocytes quercetin shows a significant induction of micronuclei in the absence and in the presence of S9. The presence of S9 compared to its absence is not significant for any of the systems used. Both in the presence and absence of S9, quercetin appears to behave as a clastogenic agent in human lymphocytes inducing a significant majority of kinetochore-negative MN.

208
Heidemann A, Tries S, Laufer S, Augustin J. STUDIES ON THE IN VITRO AND IN VIVO GENOTOXICITY OF [2,2-DIMETHYL-6-(4-CHLOROPHENYL)-7-PHENYL-2,3-DIHYDRO-1H-PYRROLIZINE-5-YL]-ACETIC ACID. Arzneimittelforschung 1995;45(4):486-90.

[2,2-Dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-1H-pyrroliz- ine-5-yl]-acetic acid (CAS 156897-06-2, ML 3000) was examined for genotoxic activity in bacteria and mammalian cells in vitro as well as in vivo. The substance did not increase gene mutation frequencies either in a bacterial system or in a cultured V79 cell line of the Chinese hamster. Both in vitro tests were conducted in the presence and absence of S9-mix. In the unscheduled DNA synthesis assay in vitro with primary rat hepatocytes, negative results were also obtained. A cytogenetic analysis of the bone marrow of male and female Wistar rats was performed. After oral application ML 3000 did not increase the number of cells with structural chromosomal aberrations. The results suggest that ML 3000 has no genotoxic potential in vitro and in vivo.

209
Anderson D, Basaran N, Blowers SD, Edwards AJ. THE EFFECT OF ANTIOXIDANTS ON BLEOMYCIN TREATMENT IN IN VITRO AND IN VIVO GENOTOXICITY ASSAYS. Mutat Res 1995;329(1):37-47.

Antioxidants are thought to be important in protecting against damage from active oxygen species. The effects of the antioxidant nutrients vitamins C and

E have been investigated after bleomycin treatment in the Salmonella typhimurium bacterial mutation assay, in the human peripheral lymphocyte chromosome aberration assay, and in the mouse micronucleus assay in peripheral blood and bone marrow cells. There were no protective effects from vitamins C and E in the bacterial mutation assay, but vitamin C and not vitamin E abolished chromosome damaging responses in human peripheral lymphocytes, and both vitamins reduced responses in micronuclei from peripheral blood cells in mice. This would suggest that in human cells in vitro and mouse cells in vivo these vitamins could have a protective role.

210
Fryxell D, Li BY, Mohanraj D, Johnson B, Ramakrishnan S. GENETIC CONSTRUCTION OF A PHOSPHORYLATION SITE IN RICIN A CHAIN:
SPECIFIC RADIOLABELING OF RECOMBINANT PROTEINS FOR LOCALIZATION AND DEGRADATION STUDIES. Biochem Biophys Res Commun 1995;210(2):253-9.

Ricin A chain was modified by the addition of the heptapeptide LRRASLG (Kemptide) and a histidine tag for bacterial expression. The mutagenized toxin was purified by nickel column and could be phosphorylated in vitro by protein kinase A as demonstrated by labeling with [gamma-32P] ATP. Kemptide-A chain could be labeled even after reassociation with ricin B chain or disulfide linkage to antibody to form an immunotoxin. The 32P label in all cases was associated only with the A chain; ricin B chain and antibody were not kinase substrates alone or after conjugation. Kemptide-immunotoxin was tested in cytotoxicity assays and used to monitor internalization of the toxin moiety after [32P] phosphorylation.

211
Leroy T, Lison D, Lauwerys R. PRELIMINARY IN VITRO INVESTIGATION INTO THE USE OF ALKALINE ELUTION ASSAY FOR THE BIOMONITORING OF HUMANS EXPOSED TO GENOTOXIC
AGENTS. Hum Exp Toxicol 1995;14(1):61-8.

1. This in vitro study was undertaken as a preliminary approach before assessing whether the alkaline elution assay can be applied to peripheral blood lymphocytes (PBL) for the monitoring of humans exposed to genotoxic agents such as polycyclic aromatic hydrocarbons (PAH). We have compared in vitro, with the aid of the alkaline elution assay, the formation and the repair of DNA single-strand breaks (ssb) induced by different genotoxic agents [gamma-irradiation, ethyl methanesulfonate (EMS), benzo<a>pyrene diol epoxide (BPDE)] on quiescent and PHA-stimulated human lymphocytes and on a fibroblast cell line. 2. Gamma-irradiation (4 Gy) induced an equivalent amount of DNA ssb in the three cell types. On the other hand, after treatment with EMS (10 mM) and BPDE (50 microM), a higher production of DNA ssb was observed in replicating cells (PHA-stimulated lymphocytes and fibroblasts) when compared with quiescent lymphocytes. 3. After gamma-irradiation, all cell types repaired more than 65% of ssb within 1 h. After treatment with EMS, we noted a deficient DNA repair capacity in quiescent lymphocytes in comparison with replicating cells. In all cell types treated with BPDE, more breaks were observed after a 2 h repair period than immediately after treatment,

demonstrating the involvement of a slow repair mechanism after BPDE treatment.(ABSTRACT TRUNCATED AT 250 WORDS)

212
Douglas GR, Gingerich JD, Soper LM. EVIDENCE FOR IN VIVO NON-MUTAGENICITY OF THE CARCINOGEN HYDRAZINE SULFATE IN TARGET TISSUES OF lacZ TRANSGENIC MICE.
Carcinogenesis 1995;16(4):801-4.

Transgenic mouse models permit the confirmation of in vitro mutagenicity in vivo without the constraints in the selection of tissues imposed by other in vivo assays. This feature is of particular importance in the determination of mutagenicity in the target tissues of carcinogens, especially those that are in vitro mutagens. Such information is critical in the determination of whether a chemical is carcinogenic via a genotoxic or non-genotoxic mechanism. Hydrazine sulfate is an in vitro mutagen that induces lung and liver tumours in mice. Transgenic mice from strain 40.6 (Mutamouse) were administered single oral doses up to a toxic concentration (400 mg/kg). No dose induced any lacZ mutations in lung, liver or bone marrow. Since the highest single dose used is higher than the cumulative dose that induced tumours in previous studies, it may be that either hydrazine sulfate is genotoxic in target tissues in vivo only when given in multiple doses or that it is a non-genotoxic carcinogen.

213
Dhillon VS, Singh J, Singh H, Kler RS. IN VITRO AND IN VIVO GENOTOXICITY OF HORMONAL DRUGS. VI. FLUOXYMESTERONE. Mutat Res 1995;342 (3-4):103-11.

Genotoxic evaluation of a commonly used synthetic steroidal androgen, fluoxymesterone, was undertaken using a combination of in vitro and in vivo assays. The clastogenic potential of fluoxymesterone was evident from the chromosome aberrations and sister chromatid exchanges induced by it in the cultured human lymphocytes and also from the increased frequencies of micronuclei and sister chromatid exchanges in bone marrow cells of mice. However, in Ames Salmonella assay both with and without S9 mix and in host-mediated assay using bacterial strains of S. typhimurium as indicator organism, fluoxymesterone did not cause any significant increase/decrease in His+ revertants.

214
Fekedu K, Parzefall W, Kronberg L, Franzen R, Schulte-Hermann R, Knasmueller S. INDUCTION OF GENOTOXIC EFFECTS BY CHLOROHYDROXYFURANONES, BYPRODUCTS OF
WATER DISINFECTION, IN E. COLI K-12 CELLS RECOVERED FROM VARIOUS ORGANS OF MICE. Environ Mol Mutag 1994;24(4):317-24.

The genotoxic effects of three chlorohydroxyfuranones (CHFs), 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), 3-chloro-4-(chloromethyl)-5-hydroxy-2(5H)furanone (CMCF) and 3,4,-dichloro-5-hydroxy-2(5H)furanone (MCA), which are formed as byproducts of water disinfection with chlorine, were investigated in bacterial differential

DNA repair assays in vitro and in animal-mediated assays in vivo. As indicators of DNA damage, E. coli K-12 strains were used that differ in their repair capacity (uvrB/recA vs. uvr+/rec+). Liquid incubation of the compounds without metabolic activation caused a pronounced reduction of the viability of the repair-deficient strain relative to the repair-proficient wild-type strain. The order of potency of genotoxic activity in vitro (dose range 0.004-10 mug/ml) was MX > CMCF > MCA. Addition of mouse S-9 mix or bovine serum albumin to the incubation mixtures resulted in an almost complete loss of the activity of all three test compounds. In the animal-mediated assays, mixtures of the indicator bacteria were injected intravenously into mice which were subsequently treated with the test compounds (200 mg/kg b.w.). Two hours later, the cells were recovered from various organs and the relative survival frequencies determined. Under these conditions, all three compounds caused pronounced genotoxic effects, MX and CMCF being stronger genotoxins than MCA. The strongest effects were consistently found in the gastrointestinal tract, but statistically significant DNA damage was also observed in indicator cells recovered from lungs, liver, spleen and kidneys. In a further experiment, the effects of lower doses of MX (4.3, 13 and 40 mg/kg) were investigated. In these experiments dose-dependent effects were measured in all organs. CMCF and MA caused only marginal effects at 40 mg/kg except in the stomach where approximately a 50% reduction of relative survival frequency was observed with CMCF. The results of these animal-mediated assays indicate that (i) all three CHFs cause genotoxic effects in the living animal, and (ii) the potencies of the three compounds observed under in vivo conditions are not commensurate with their extremely high activities measured in vitro. One possible explanation for the weaker responses observed in the animal-mediated assays might be that CHFs are inactivated by nonspecific protein binding.

215
Daugel'-Dauge NO, Durnev AD, Kulakova AV, Seredenin SB, Velichkovskii BT. [CORPUSCULAR MUTAGENESIS AND ITS PREVENTION.] Vestn Ross Akad Med Nauk 1995;1:29-38. (Rus)

The carcinogenic and mutagenic activity of dust containing chrysotile-asbestos and zeolites, as well as the role of active oxygen species in their cytotoxic and mutagenic actions are discussed. Superoxide dismutase (50 mg/ml) was demonstrated to prevent the mutagenic effects of chrysotile-asbestos and latex, catalase (20 mg/ml) to prevent the same of zeolites in experiments on cultured human whole blood. The intraperitoneal administration of dusts of chrysotile-asbestos and zeolites in a dose of 50 mg/kg to C57B1/6 mice was found to elevate the count of cells with chromosomal aberrations in the peritoneal liquid and bone marrow cells of mice, which was dependent on dust exposure time. It was revealed that ascorbic acid, rutin, chemically modified flavonoid of Scutellaria Baicalensis Georgy, drugs such as bemitil and thomersol in the broad range of concentrations (10(-7)-10(-3) M) decreased or completely reduced the clustogenic action of zeolites and chrysotile-asbestos on cultured human whole blood. The ability of bemitil (1.8-19 mg/kg) rather than the others to prevent the mutagenic effect of chrysotile-asbestos was confirmed by the method of recording chromosomal aberrations in the cells of peritoneal liquid and bone marrow in mice. The findings suggest that the mutagenic effects of the corpuscular xenobiotics

under study are mediated by active oxygen species and that the use of the models in vitro and in vivo is adequate for investigations into corpuscular mutagenesis. Based on their own data and literature data, the authors have defined possible lines of further research of corpuscular mutagenesis.

216
Donnelly KC, Safe SH, Randerath K, Randerath E. BIOSASSY-BASED RISK ASSESSMENT OF COMPLEX MIXTURES. J Hazard Mater 1995; 41(2-3):341-50.

In order to compare a std. chem.-based risk assessment with in vitro genotoxicity assays, two complex environmental mixts. from a wood-preserving site were analyzed in the Salmonella/microsome and E. coli prophage induction assays. Using GC/MS, sample 003 was found to contain relatively low levels of polycyclic arom. hydrocarbons (PAHs) and elevated levels of polychlorinated dibenzo-p-dioxins (PCDDs), while sample 005 had higher levels of PAHs and relatively low levels of PCDDs. The complex mixts. were sequentially extd. with methylene chloride and methanol for anal. in Salmonella, or extd. with a 1:1 hexane:acetone mixt. for anal. in the E. coli prophage induction assay. At a dose of 1.0 mg/plate in Salmonella strain TA98 with metabolic activation, the methanol ext. of sample 003 induced 197 net revertants, while sample 005 induced 436 net revertants. In the prophage induction assay, with activation, the hexane:acetone ext. of sample 003 induced a genotoxic response that was slightly lower than that obsd. with sample 005. The estd. incremental carcinogenic risk for ingestion of PAHs was 1.5E-3 for sample 003, while for sample 005 the estd. risk was 1.5E-2. Thus, the sample which induced the max. response in both bioassays also had the highest estd. cancer risk. However, the frequency of PAH-DNA adducts in both skin and liver tissues was appreciably higher with sample 003 than with sample 005. A combined testing protocol, using both biol. and chem. anal., therefore provides more accurate information from which to assess risk than the use of either method alone.

217
De Deyne PG, Kirsch-Volders M. IN VITRO EFFECTS OF THERAPEUTIC ULTRASOUND ON THE NUCLEUS OF HUMAN FIBROBLASTS. Phys Ther 1995;75(7):629-34.

The purpose of this investigation was to examine the cytological effects of therapeutic ultrasound on human fibroblasts. MATERIAL AND METHODS. Using an in vitro approach, the number of cells recovered, the morphology of chromosomes, and the presence of mitotic spindles were studied after sonication of human fibroblasts in culture. The ultrasound output had a frequency of 1 MHz and was delivered in a pulse mode of 2 milliseconds "on" and 8 milliseconds "off." Sonication was given for 0, 30, 60, and 90 seconds. RESULTS. There was a time-dependent decrease in number of cells recovered, a fourfold increase in mitotic index in the cells that survived the treatment, and a nearly eightfold increase of chromosomal aberrations with loss of mitotic spindles. CONCLUSION AND DISCUSSION. The dose-dependent lytic effect of nonthermal ultrasound could result in fractionation of cells, which might facilitate phagocytosis during chronic inflammation. In addition, we observed an increase in chromosomal aberrations and a loss of mitotic spindles as a result of an extremely short ultrasound treatment (30 seconds). Whether these alterations are mutagenic or not warrants further study. It is possible that in our in vitro experiments,

the ultrasound received was greater than would occur in a clinical situation.

218
Wilmer JL, Luster MI. CHEMICAL INDUCTION OF INTERLEUKIN-8, A PROINFLAMMATORY CHEMOKINE, IN HUMAN EPIDERMAL KERATINOCYTE CULTURES AND ITS RELATION TO CYTOGENETIC TOXICITY. Cell Biol Toxicol 1995;11(1):37-50.

Tumor promoters, proinflammatory cytokines, endotoxins, and protein synthesis inhibitors can modulate cell cycle kinetics of various cell types, stimulate production of reactive oxygen species, and induce keratinocytes to produce interleukin-8 (IL-8), a potent chemotactant for polymorphonuclear neutrophils and T lymphocytes. The aim of this study was to determine whether perturbations of cytogenetic responses correlated with the induction of IL-8 expression. Cultures of primary human keratinocytes were grown in serum-free medium with 5 mumol/L bromodeoxyuridine to label DNA and exposed either to phorbol-13-myristate-12-acetate (PMA) (0.0001-100 ng/ml), cycloheximide (CHX) (0.01-50 mug), lipopolysaccharide (0.1-100 mug/ml), tumor necrosis factor-alpha (TNFalpha) (3.13-50 ng/ml), or interleukin-1alpha (IL-1alpha) (1-182 pg/ml). Metaphase chromosome preparations were stained by a fluorescence-plus-Giemsa technique to differentiate sister chromatids. For IL-8 production, keratin grown to 70% confluency and then exposed to chemicals for 24 h. Immunoreactive IL-8 was quantitated from the supernatants by ELISA. With the exception of benzo(a)pyrene used as a positive control, none of the agents induced sister chromatid exchanges. However, PMA and TNFalpha induced IL-8 production that coincided with significant cell cycle inhibition. IL-1alpha had no effect on cytogenetic endpoints, yet stimulated a 6.3-fold increase in IL-8. CHX inhibited cell cycle progression and mitotic activity at concentrations that were 200 times lower than required for IL-8 induction; however, puromycin (0.31-10 mug/ml), another protein synthesis inhibitor, did not induce IL-8. At all concentrations tested, TNFalpha reduced the mitotic index byuced a flat, albeit large, IL-8 response at concentrations : 12.5 ng/ml. These agent-specific response patterns suggest that induction of IL-8 production is not always the inevitable result of cell cycle perturbations or genetic damage.

219
Cravino V, Kropko ML, Rothwell CE, Hovey CA, Theiss JC. THE GENOTOXICITY PROFILE OF ATORVASTATIN, A NEW DRUG IN THE TREATMENT OF HYPERCHOLESTEROLEMIA.
Mutat Res 1995;343(2,3):95-107.

While HMG-CoA reductase inhibitors such as fluvastatin, lovastatin, pravastatin and simvastatin demonstrate lack of in vitro and in vivo mutagenicity and clastogenicity in bacterial and mammalian cells, long term rodent carcinogenicity studies resulted in an increased incidence in neoplasms at high doses. These effects may be attributable to an exaggeration of the desired biochem. effect of the drug and/or a tumor promoting effect. The genotoxicity of atorvastatin, a newly developed HMG-CoA reductase inhibitor, was evaluated in a variety of test systems. In bacterial mutagenicity tests, the E. coli tester strain WP2(uvrA) and S. typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to concns. of atorvastatin as high as

5000 mug/plate both in the absence (S9-) and presence (S9+) of metabolic activation. Atorvastatin was not mutagenic in either E. coli or S. typhimurium. Chinese hamster lung V79 cell cultures were exposed to atorvastatin at concns. of 50-300 mug/mL (S9-) and 100-300 mug/mL (S9+) and structural chromosome aberrations were assessed. Mutation at the hgprt locus was assessed at concns. of 100-300 mug/mL (S9-) and 150-275 mug/mL (S9+). Atorvastatin was neither mutagenic nor clastogenic in the absence or presence of S9. The lack of in vitro genotoxicity was corroborated in vivo in a mouse micronucleus study in which single oral doses of atorvastatin were administered to male and female CD-1 mice at 1, 2500, or 5000 mg/kg. No biol. significant increases in the frequency of micronucleated polychromatic erythrocytes in bone marrow at 24, 48, or 72 h postdosing were obsd. Thus, atorvastatin, as with the other tested HMG-CoA reductase inhibitors, is not genotoxic.

220
Takahashi N, Shibahara T, Shiragiku T, Kanbe T, Kanbe K, Sugawara M, Yamashita S. REDUCTION OF IN VITRO CLASTOGENICITY INDUCED BY THE MIXTURE OF OPTICAL ISOMERS OF NADIFLOXACIN DURING STORAGE. Arzneimittelforschung 1995;45(2):195-7.

The fluoroquinolone antibacterial agent, nadifloxacin (NDFX, CAS 124858-35-1), is a racemic compound. The storage effect on the in vitro clastogenicity of a solution of the racemic compound and a mixture solution of the optical isomers of NDFX, prepared by mixing equal amounts of S- and R-enantiomers, was investigated. The potential of NDFX and the enantiomer mixture, prepared from equal amounts of each S- and R-enantiomer, to induce chromosomal aberrations in vitro was investigated in cultured fibroblasts derived from Chinese hamster lung cells immediately, 2 and 4 weeks after preparation of the test solutions (stored at 20 degrees C, protected from light) using 24 h of continuous treatment method. In the results, NDFX did not significantly increase the incidence of chromosomal aberrations at 200 micrograms/ml regardless of the storage period. On the other hand, the mixture significantly increased the incidence of chromosomal aberrations at 200 micrograms/ml immediately after preparation to an extent similar to that of S-enantiomer alone, but the mixture did not do so after 2 and 4 weeks of storage. Neither S- nor R-enantiomer changed the chromosomal aberration inducibility during storage. The content and optical purity of the test substances in each test solution also did not change during storage. These facts suggested that the molecular condition of each optical isomer in the mixture solution became equivalent to that in the racemic solution during storage periods.

221
Suzuki O, Ishimura K, Takahashi T, Miyauchi S. [IN VITRO CHROMOSOME ABERRATION TEST OF SODIUM HYALURONATE IN CULTURED MAMMALIAN CELLS.] Oyo Yakuri 1995;50(1):73-7. (Jpn)

As part of a mutagenicity testing program, sodium hyaluronate (Na-HA) with a mol. wt. of 276.times.104 was examd. for its clastogenicity by using cultured Chinese hamster lung fibroblast cells. Concns. of 1,250, 2,500 and 5,000 mug/mL were tested with and without metabolic activation. Examn. of 200

mitosis per concn. for chromosome aberrations showed that Na-HA did not differ significantly from the neg. control. It is concluded that Na-HA with a mol. wt. of 276.times.104 is not a clastogen under the conditions of the expt.

222
Wening JV, Marquardt H, Katzer A, Jungbluth KH, Marquardt H. CYTOTOXICITY AND MUTAGENICITY OF KEVLAR: AN IN VITRO EVALUATION. Biomaterials 1995;16(4):337-40.

Toxicity and mutagenicity of Kevlar 49 (PPPT; poly-para-phenylene-terephthalamide) was tested in six strains of Salmonella typhimurium (Ames test; TA97, TA98, TA100, TA102, TA1535, TA1537) with and without an external metabolic activation system (S9), as well as in a mammalian cell mutagenesis assay using V79 Chinese hamster cells. For the Ames test, liquid preincubation, which is considered particularly sensitive, was used. The cells were incubated for 24 h at a temperature of 37 degrees C either directly with Kevlar49 or with ethanol- or chloroform-extracted Kevlar49. The experiments were performed at least twice. The Ames test with six different Salmonella typhimurium strains featuring either base pair substitution or frameshift mutations revealed no cytotoxic or mutagenic activity of Kevlar49. In the mammalian cell mutagenesis assay, using 8-azaguanine (AG) as a selective agent, Kevlar49 was also devoid of cytotoxic or mutagenic activity. Both tests have to be regarded as an initial exploratory screening due to the chosen testing conditions and should be supplemented by tests at different temperatures.

223
Jacobson-Kram D, Rosenthal SL. MOLECULAR AND GENETIC TOXICOLOGYOF 1,3-BUTADIENE. Mutat Res 1995;339(2):121-30.

During the last 9 years, there have been many studies published concerning the mutagenic potential of butadiene in mammalian systems, including alterations at the molecular level. Butadiene has tested positive in several mouse in vivo and in vitro assays, but has generally tested negative in rat studies. Most of these studies are cytogenetic and include positive data in mice for chromosomal aberrations, micronucleus formation, and sister chromatid exchanges. Butadiene also induces mutations in lung, spleen, and bone marrow of transgenic mice. The positive bone marrow cytogenetic and transgenic data may be significant in view of the increased lymphohematopoietic malignancies observed in mice and probably in humans. In addition, butadiene causes mutations in the K-ras protooncogene and in the p53 tumor suppressor gene in mouse studies. Mutations in these genes are associated with oncogenesis in humans as well as in rodents. Also, positive mutagenicity data have been obtained in a pilot study of workers exposed to butadiene. Positive dominant lethal studies in rodents suggest that exposure to butadiene can result in germ cell mutation and heritable risk. These mutagenicity and molecular data suggest that butadiene is both a somatic and germ cell mutagen in mammals, possibly including humans.

224
Guo YJ, Lu SX, Liang YY. [ALTERATIONS OF ONCOGENES IN HUMAN FETAL ESOPHAGEAL

EPITHELIUM INDUCED BY N-METHYLBENZYLNITROSAMINE (NMBzA).] Chunghua Chung Liu Tsa Chih 1994;16(6):407-10. (Chi)

Epidemiological investigation showed that N-methylbenzylnitrosamine (NMBzA) has been associated with increased incidence of esophageal cancer (EC) in Linxian county, a high incidence area. In present study, our results indicate that NMBzA can induce amplification and over-expression of EGFr gene in human fetal esophageal epithelium (HFE) treated with NMBzA for 24 hours as shown by southern blot assay and immunohistochemistry. The papillary hyperplasia was induced in HFEs that cultured with NMBzA for 1 to 3 weeks. Amplification of c-myc and int-2 gene in HFEs treated by NMBzA for 1 week and 3 weeks was found, respectively. Deletions of p53 and Rb gene were found in human fetal esophageal carcinomas induced by NMBzA. Overexpression of p53 protein in human fetal esophageal carcinomas detected by immunohistochemical methods indicates that p53 gene mutation(s) may be occured. The HFE explants treated in vitro with NMBzA for 3 weeks were inoculated subcutanously into balb/c nude mice. No tumor was found in 5 months after inoculation, suggesting that only changes of oncogene(s) are insufficient to induce full transformation. Other genetic alterations (such as functional inactivation of Rb or/and p53 tumor suppressor genes) may be necessary in the further progression of malignant lesions.

225
Tateno H, Kamiguchi Y. APPLICATION OF CRYOPRESERVED GOLDEN HAMSTER OOCYTES TO
IN VITRO GENOTOXICITY ASSAYS FOR HUMAN SPERM CHROMOSOMES. Environ Mol Mutagen
1995;25(3):263-5.

226
Turk PW, Laayoun A, Smith SS, Weitzman SA. DNA ADDUCT
8-HYDROXYL-2'-DEOXYGUANOSINE (8-HYDRONYGUANINE) AFFECTS FUNCTION OF HUMAN DNA
METHYLTRANSFERASE. Carcinogenesis 1995;16(5):1253-5 .

8-Hydroxyl-2'-deoxyguanosine (also referred to as 8-hydroxyguanine [8-OH-dG] or 7,8-dihydro-8-oxoguanine), a common DNA adduct resulting from injury to DNA via reactive oxygen species, affects the in vitro methylation of nearby cytosine moieties by the human DNA methyltransferase. The exact position of 8-OH-deoxyguanosine relative to a CpG dinucleotide appears important to this effect. Our data indicate that 8-OH-deoxyguanosine diminishes the ability of the methyltransferase to methylate a target cytosine when the 8-OH-deoxyguanosine is one or two nucleotides 3' from the cytosine, on the same strand. On the other hand 8-OH-deoxyguanosine does not diminish the ability of the enzyme to respond to a methyl director (5-methylcytosine) when the 8-OH-deoxyguanosine is on the same strand but one or two nucleotides 3' from the methyl director. Differences in methylation rates as great as 13-fold have been detected using various 8-OH-deoxyguanosine-containing oligonucleotides as substrates in methylation assays. Our findings suggest that oxidative damage of parental strand guanines would permit normal copying of methylation patterns through maintenance methylation, while oxidative damage of guanines in the nascent strand DNA would inhibit such methylation.

227
Erexson GL, Bryant MF, Kwanyuen P, Kligerman AD. BLEOMYCIN SULFATE-INDUCED MICRONUCLEI IN HUMAN, RAT, AND MOUSE PERIPHERAL BLOOD LYMPHOCYTES. Environ Mol
Mutagenesis 1995;5(1):31-6.

The sensitivity to micronucleus (MN) induction of human, mouse, and rat peripheral blood lymphocytes (PBLs) exposed to bleomycin sulfate (BLM) in vitro was compared in cytochalasin B-induced binucleated (BN) cells. For the PBLs of each species, either 0, 5, 10, 20, 40, 60, 80, or 160 mug/ml BLM was added to 5 ml aliquots of whole blood for 4 hr at 37~C in a 5% CO2 atmosphere. Leukocytes were isolated on a density gradient and cultured in the presence of phytohemagglutinin to stimulate blastogenesis, and cytochalasin B was added to each culture at 21 hr postinitiation to prevent cytokinesis. A total of 4,000 BNs/concentration/species was analyzed for MN in two independent experiments. In addition, multiple-MN-BNs were quantitated, and the nucleation index was determined. Significant increases both in total MN-BNs and multiple MN-BNs were observed at all concentrations in all species. All three species' concentration-response curves gave good fits (r2 values from 0.87 to 0.95) to either a linear or a square root model (y = mx + b or y = m(x)0.5 + b, respectively; where y = the percentage of MN-BN, m is the slope, and b is the y-intercept). The MN induction in the human and rat PBLs was not statistically different, but both were significantly less sensitive than the response shown by the BLM-exposed mouse PBLs. This difference in MN susceptibility was observed only at BLM test concentrations : 20 mug/ml. The nucleation index was significantly decreased in all species at either 80 or 160 mug/ml.

228
Hinton A Jr, Hume ME. SYNERGISM OF LACTATE AND SUCCINATE AS METABOLITES UTILIZED BY VEILLONELLA TO INHIBIT THE GROWTH OF SALMONELLA TYPHIMURIUM AND
SALMONELLA ENTERITIDIS IN VITRO. Avian Dis 1995;39(2):309-16.

The inhibition of salmonellae growth by a Veillonella bacterium isolated from the cecal contents of adult chickens was examined. The Veillonella isolate was grown on an agar medium supplemented with 175 mumol of lactate or succinate/ml. Either 0, 100, 125, 150, or 175 mumol of succinate/ml was added to the lactate medium; either 0, 100, 125, 150, or 175 mumol of lactate/ml was added to the succinate medium; and the pH of all media was adjusted to 6.0. Agar overlays of Veillonella cultures grown on the media were inoculated with Salmonella typhimurium or S. enteritidis. The largest zones of inhibition of salmonellae growth were produced by Veillonella cultures grown on medium supplemented with 175 mumol/ml of both lactate and succinate. The widths of the zones of inhibition decreased as the concentration of lactate was reduced in the succinate medium and as the concentration of succinate was reduced in the lactate medium. Analyses of lactate broth and succinate broth inoculated with Veillonella indicated that inhibition of salmonellae growth on the agar media was related to the production of volatile fatty acids by Veillonella, the presence of residual succinate in the media, and the final pH of the media.

229
Mothersill C. HUMAN ESOPHAGEAL CULTURE. Methods Mol Biol 1995;43:75-9.

230
Jorritsma U, Cornet M, Van Hummelen P, Bolt HM, Vercruysse A, Kirsch-Volders M, Rogiers V. COMPARATIVE MUTAGENICITY OF 2-METHYLPROPENE (ISOBUTENE), ITS EPOXIDE 2-METHYL-1,2-EPOXYPROPANE AND PROPYLENE OXIDE IN THE IN VITRO MICRONUCLEUS TEST USING HUMAN LYMPHOCYTES. Mutagenesis 1995;10(2):101-4.

2-Methylpropene (isobutene), a gaseous compound widely used in chemical industries, is metabolized to the epoxide 2-methyl-1,2-epoxypropane. The parent compound has previously been shown to be non-mutagenic in a modified Ames test, whereas the epoxide metabolite gave a positive result. In this study, both compounds have been tested in the in vitro micronucleus test using human lymphocytes. Propylene oxide, a well known mutagenic compound, served as a positive control. It was found that 2-methylpropene had no mutagenic effect, whereas its epoxide induced a statistically significant dose-dependent increase in the number of micronuclei. The effect observed was comparable with

that obtained for propylene oxide.

IMMUNOTOXICITY
231
Honda T, Miwatani T, Yabushita Y, Koike N, Okada K. A NOVEL METHOD TO CHEMICALLY IMMOBILIZE ANTIBODY ON NYLON AND ITS APPLICATION TO THE RAPID AND
DIFFERENTIAL DETECTION OF TWO VIBRIO PARAHAEMOLYTICUS TOXINS IN A MODIFIED ENZYME-LINKED IMMUNOSORBENT ASSAY. Clin Diag Lab Immunol 1995;2(2):177-81.

A new method of chemically immobilizing antibody on nylon was developed. The method consists of serial treatments with HCl, polyethylene imine, and maleic anhydride methylvinyl ether copolymer, which resulted in the stable immobilization of sufficient amounts of antibodies on nylon. This principle was used to differentially detect two immunologically related but nonidentical hemolysins (thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH)) of Vibrio parahaemolyticus in a modified enzyme-linked immunosorbent assay with antibodies immobilized on nylon slips (NSIT). The results (dark purple color on nylon slips) were easily evaluated by the naked eye. The results with NSIT were compatible with those obtained by using DNA probes or a conventional bacterial culture test, not only with cultured specimens but also with clinical specimens (diarrheal stool samples). Furthermore, the NSIT differentially detected TDH and TRH in a single test. The antibody immobilization method developed here is applicable to various immunological detection methods and may improve their sensitivity and specificity.

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Nicklin S. IMMUNE FUNCTION ASSAYS. Methods Mol Biol, 1995;43:245-56.

233
House RV, Thomas PT, Bhargava HN. SELECTIVE MODULATION OF IMMUNE FUNCTION RESULTING FROM IN VITRO EXPOSURE TO METHYLENEDIOXYMETHAMPHETAMINE (ECSTASY).
Toxicology 1995; 96(1):59-69.

Abuse of illicit analogs of methamphetamine (i.e., 'designer drugs') represents a growing problem. One of the most popular methamphetamine analogs is (:)-3,4- ethylenedioxymethamphetamine (MDMA), commonly known as Ecstasy. The authors demonstrated previously that in vitro exposure to methamphetamine results in modulation of immune functional parameters necessary for host defense. The current study was performed to assess the potential direct (in vitro) immunomodulatory effect of exposure to a modified methamphetamine. Splenocytes or peritoneal macrophages from B6C3F1 mice were cultured in vitro at MDMA concentrations of 0.0001-100 muM. T-cell regulatory function was assessed by anti-CD3-mediated production of IL-2 and IL-4, B-cell function was assessed by quantitating cellular proliferation, natural immunity was assessed by quantitating natural killer (NK) cell activity, T-cell effector function was evaluated as a unction of cytotoxic T-lymphocyte (CTL) activity, and macrophage function was assessed by IL-6 tumor necrosis factor (TNF) production. In vitro exposure to MDMA had no effect on B-cell proliferation at any concentration tested. In comparison, in the absence of direct cellular toxicity, production of IL-2 was enhanced at concentrations as low as 0.0001 muM. IL-4 production was not affected by exposure to any concentration of MDMA examined, suggesting a differential alteration in T-helper cell function by this compound. Basal and augmented NK cell function were enhanced at MDMA concentrations between 0.0001 and 1.0 muM when examined at an effector:target ratio of 100:1. CTL induction was significantly suppressed at a concentration of 100 muM. Finally, macrophage production of TNF was slightly suppressed at 10 and 100 muM MDMA, although this inhibition was not statistically significant.

234
Jeong TC, Matulka RA, Jordan D, Yang KH, Holsapple MP. ROLE OF METABOLISM IN COCAINE-INDUCED IN IMMUNOSUPPRESSION IN SPLENOCTYE CULTURES FROM B6C3F1 FEMALE
MICE. Immunopharmacology 1995;29(1): 37-46.

Cocaine has been reported to directly suppress the in vitro immune responses at very high concentrations. In the present study, the possible role of metabolism in cocaine-induced immunosuppression was investigated in splenocyte cultures isolated from B6C3F1 female mice. Since cocaine can be metabolized by both esterase and P-450 monooxygenase, we studied the direct effects of cocaine, benzoylecgonine and norcocaine on the in vitro T-dependent antibody response to SRBC. Direct exposure to cocaine only produced a modest (30%) but nonsignificant suppression of the antibody response, while benzoylecgonine, a primary product of metabolism by the esterase pathway, was devoid of activity. In contrast, direct exposure to norcocaine, the initial product of N-demethylation by the P-450 pathway, produced significant suppression at concentrations greater than or equal to 10 muM. Similar results were observed in studies measuring LPS and Con A mitogenicity. Furthermore, a significant

suppression was observed when splenocytes were preincubated for 1 h with 1 mM cocaine in the presence of liver S-9 fractions isolated from phenobarbital-induced mice. Meanwhile, no suppression was obtained when splenocytes were preincubated in the presence of untreated S-9 fractions. To characterize the mechanism of our results, the capacity of both untreated and phenobarbital-induced microsomes to produce formaldehyde from cocaine was compared. The Ar-demethylation of cocaine was NADPH-dependent and phenobarbital-induced microsomes produced approx. 6-times higher amounts of formaldehyde, indicating a greater portion of cocaine could be metabolized through the P-450 pathway to its toxic metabolites. Finally, because benzoylecgonine shares with cocaine the presence of a methyl group on the tropane nitrogen, we also compared the ability of N-demethylation from cocaine and benzoylecgonine in mouse liver microsomes. Our results indicated that benzoylecgonine could not be demethylated as determined by a failure to generate any formaldehyde. These results offer further support that the N-demethylation pathway is a critical step to cause its immunotoxicity.

235
Kuehn U, Lempertz U, Knop J, Becker D. A NEW METHOD FOR PHENOTYPING PROLIFERATING CELL NUCLEAR ANTIGEN POSITIVE CELLS USING FLOW CYTOMETRY: IMPLICATIONS FOR ANALYSIS OF THE IMMUNE RESPONSE IN VIVO. J Immunol Methods 1995;179(2):215-22.

The incorporation of radioactive nucleotides into newly synthesized DNA has been established as a standard method for the detection of proliferation in eucaryotic cells. Unfortunately the use of this method makes it harder to obtain information on the phenotype of proliferating cells in mixed cell populations. For this reason we established a flow-cytometric approach employing a monoclonal antibody specific for murine as well as human proliferating cell nuclear antigen (PCNA) and a double labeling technique for detection of cell membrane-expressed phenotypic markers. The efficiency of this immunostaining procedure was confirmed by simultaneous and highly specific detection of PCNA in nuclear structures as well as cell membrane-expressed antigens using cytological techniques. In vitro experiments with mitogen- and alloantigen-stimulated murine lymph node cells (LNC) and human peripheral blood mononuclear eukocytes (PBML) revealed a good correlation of total (3H)thymidine incorporation into DNA and expression of PCNA. For the analysis of proliferating cells activated in vivo the method was employed to evaluate the local lymph node assay which assesses the allergenicity of small chemicals. LNC prepared from the cervical lymph nodes of mice treated on 4 consecutive days with sensitizing concentrations of the contact allergens oxazolone, TNCB and DNFB as well as the irritants benzoic acid and SLS in comparison to the solvent control showed a dramatic increase in the total amount of proliferating cells for contact allergen-treated animals in comparison to the solvent control and irritant-treated mice. In addition a detailed phenotyping of the proliferating cell populations was possible. This approach offers an easy to perform, non-radioactive method for the assessment of proliferation of murine as well as human leukocytes in vitro and especially in vivo and will be of great advantage for situations where the phenotype of proliferating cellular subsets in heterogeneous populations is of interest.

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Garssen J, Van der Vliet H, De Klerk A, Goettsch W, Dormans JA, Bruggeman CA, Osterhaus AD, Van Loveren H. A RAT CYTOMEGALOVIRUS INFECTION MODEL AS A TOOL FOR IMMUNOTOXICITY TESTING. Eur J Pharmacol 1995;292 (3-4):223-31.

A rat cytomegalovirus infection model for use in immunotoxicity testing has been developed. In resistance against viruses, natural killer cells and cytotoxic T-cells play an important role. Therefore, this model complements other rat host resistance models for immunotoxicity testing, i.e. existing bacterial and parasitic infection models in which cytotoxic T-cells and natural killer cells play a minor role. Host resistance against cytomegalovirus infections in the rat was determined by titrating infectious virus levels in organs after cytomegalovirus infection in an in vitro infectivity test denoted as the Plaque Forming Unit (PFU) Test. In this test, homogenates of different organs were investigated for infectious virus titers on rat embryonic cell monolayers. We demonstrated that in the salivary gland, the major target organ for rat cytomegalovirus, virus was detectable from 8 days onward after intraperitoneal infection. To show that this model is suitable for the detection of immunotoxicity four different methods for immunosuppression were investigated: 1. gamma-irradiation, 2. congenitally athymic rats, 3. chemically induced immunosuppression, 4. ultraviolet-B (UVB) irradiation. Rat cytomegalovirus titers in the salivary glands of irradiated (500 rad 1 day prior to infection) or congenitally athymic rats were significantly increased as compared to non-irradiated rats and euthymic control rats respectively. In TOX-Wistar rats, given 20 or 80 mg bis(tri-n-butyltin)oxide (TBTO) per kg food beginning 6 weeks before cytomegalovirus infection, a regimen known to have immunotoxic effects, cytomegalovirus titers in the salivary glands were significantly increased as compared to non-TBTO-treated cytomegalovirus infected rats.(ABSTRACT TRUNCATED AT 250 WORDS)

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Yokozeki H, Katayama I, Nishioka K. EXPERIMENTAL STUDY FOR THE DEVELOPMENT OF AN IN VITRO TEST FOR CONTACT ALLERGENS: 1. PRIMARY ACTIVATION OF HAPTEN-CONJUGATED EPIDERMAL CELLS. Int Arch Allergy Immunol 1995;106(4):394-400.

We conducted a study on the primary in vitro activation of T cells from nonsensitized mice by using hapten-conjugated Pam 212 cells (keratinocyte cell line). Furthermore, we attempted to develop a simple, quantitative in vitro test to assess the sensitizing potency of contact allergens and applied it to determine the stimulation index (SI) of various chemicals with known degrees of sensitizing potency. Monolayered Pam 212 cells were incubated with a variety of chemicals exhibiting allergenic potential. Washed and fixed T cells depleted of autoreactive T cells and macrophages from spleens of nonsensitized Balb/c mice were cocultured for 5 days with those monolayered Pam cells conjugated with chemicals. They were then harvested and restimulated with mitomycin-C-treated spleen cells conjugated with chemicals in 96-well culture plates to inhibit the proliferation of stimulator cells. We evaluated the

sensitizing potency of the following chemicals: oxazolone, TNBS, DNFB and FITC (strong sensitizers); p-phenylendiamine (p-PD), nickel chloride and potassium dichromate (potent sensitizers); betamethasone and budesonide (corticosteroids), and methyl salicylate (MS) as an irritant. T cells sensitized in vitro with TNP-Pam cells and macrophages demonstrated antigen-specific proliferation when restimulated in vitro with mitomycin-C-treated TNP-spleen cells. Subcutaneous injection of these T cells induced contact sensitivity in vivo in an antigen-specific fashion. While T' cells cocultured with TNP-3T3 could not be activated even in the presence of macrophages. The SI of strong sensitizers was about 4.00 and that of p-PD was 2.36. The SIs of budesonide, betamethasone and MS were 1.62, 0.98 and 1.21, respectively. These results suggest that this method of producing primary hapten-specific T cell activation demonstrated its potential to become an in vitro test for contact allergens in the future.

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Koh WS, Yang KH, Jeong TC, Delany B, Kaminski NE. 2-ACETYLAMINO-FLUORENE INHIBITS THE ACTIVATION OF IMMUNE RESPONSES BY BLOCKING CELL CYCLE PROGRESSION
AT G1 PHASE. Arch Toxicol 1995;69(5): 350-6.

2-Acetylaminofluorene (AAF) inhibited in a dose dependent manner mouse spleen cell blastogenesis in response to phorbol 12-myristate 13-acetate (PMA)/Ionomycin (Io) activation, the T-cell lectin, concanavalin A (Con A), and following stimulation by alloantigens as measured by the mixed lymphocyte response (MLR). AAF also markedly suppressed the T-cell dependent antibody forming cell (AFC) response to sRBC. AAF was most inhibitory on both the sRBC IgM AFC response and Con A stimulated proliferation when added during the first 24 h following initiation of culture. Direct addition of high concentrations of AAF (100 muM) to spleen cell cultures at 48 h following Con A stimulation produced a very modest inhibition (<20%) of T-cell proliferation as compared to 90% when added at the time cultures were initiated. Similarly, AAF (75 and 100 muM) produced a greater than 80% inhibition of the in vitro AFC response when spleen cells were sensitized with antigen in presence of AAF. In contrast, no inhibition of the IgM AFC response was produced when AAF (75 muM) was added to spleen cell cultures 48 or 72 h after antigen sensitization. Con A-triggered cell-cycle progression was attenuated at the G1 stage by the addition of AAF (50 and 100 muM) with no inhibition of S to G2/M phase transition. These results suggest that the mechanism of AAF-mediated immune suppression is through a blockade of cell cycle progression from G1 to S phase.

239
Post J, Vooys WC, de Gast GC, Bast BJ. COMPARISON OF VARIOUS IN VITRO ASSAYS FOR EFFICACY SCREENING OF IMMUNOTOXINS. Leuk Res 1995;19(4):241-7.

Before using immunotoxins in vivo, their efficacy is evaluated in in vitro assays. In this study we compare six different assays for the evaluation of immunotoxins: protein and DNA synthesis inhibition assay, chromium release assay, cell line colony assay, limiting diln. assay and clonogenic assay. All

assays except the chromium release assay show specificity of the immunotoxins in appropriate concns. The protein and DNA synthesis inhibition assays are easy to perform and, therefore, suitable for initial screening, while the clonogenic assay seems to be the best one for immunotoxin efficacy detn.

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House RV, Thomas PT, Bhargava HN. IN VITRO EVALUATION OF FENTANYL AND MEPERIDINE FOR IMMUNOMODULATORY ACTIVITY. Immunol Lett 1995; 46(1,2):117-24.

Exposure to drugs, either ethical pharmaceuticals or illicit street drugs, often results in medical complications, including alterations in the immune system. Among the drugs assocd. with immunomodulatory potential are the analgesics fentanyl and meperidine. The purpose of this study was to det. the potential of these drugs to alter immunol. parameters subsequent to in vitro exposure at a range of concns. This potential immunotoxicity was assessed using a series of in vitro assays measuring B-lymphocyte proliferation, cytokine prodn. by T-helper lymphocytes, T-lymphocyte cytolytic function, natural killer (NK) cell function, and macrophage function. Exposure to these

analgesics was assocd. with a differential suppression of interleukin-4 prodn. by T-cells, as well as a more generalized suppression of cytokine prodn. by macrophages. In addn., T-cell cytolytic activity was suppressed at high drug concns. B-cell proliferation and NK cell activity were also inhibited, but to a lesser degree than noted with T-cell function. Addn. of naltrexone to the cultures did not reverse these alterations in immune function, suggesting that these changes are not mediated via opioid receptors.

NEUROTOXICITY
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Ennis MD, Stjernloef P, Hoffman RL, Ghazal NB, Smith MW, Svensson K, Wikstroem H, Haadsma-Svensson SR, Lin C. STRUCTURE-ACTIVITY RELATIONSHIPS IN THE 8-AMINO-6,7,8,9-TETRAHYDRO-3H-BENZ[E]INDOLE RING SYSTEM. Part 2: EFFECT OF 8-AMINO NITROGEN SUBSTITUTION ON SEROTONIN RECEPTOR BINDING AND PHARMACOLOGY.
J Med Chem 1995;38(12):2217-30.

A series of analogs of the potent and selective 5-HT1A agonist 8-(di-n-propylamino)-6,7,8,9-tetrahydro-3H-benz[e]indole-1-carbal- dehyde (OSU191) was prepd. in which the dipropylamino group was modified to bear a variety of substituents. These compds. were evaluated for both in vitro and in vivo effects, including the establishment of a receptor binding profile for these analogs at the 5-HT1A, dopamine D-2, dopamine D-3, 5-HT1Dalpha, and 5-HT1Dbeta sites. Several of the analogs were evaluated for their biochem. effects in reserpinized rats, specifically with regard to in vivo changes in brain levels of 5-HTP and DOPA. Nearly all of the compds. prepd. for this study were exceedingly potent at the 5-HT1A receptor, although most also displayed significant affinity for the dopamine D-2 receptor. A strong preference for the 5-HT1Dalpha over the 5-HT1Dbeta receptor was also apparent. An analog bearing a butylglutarimide side chain, S-7k, was extremely selective for the 5-HT1A receptor. Although this compd. possessed a Ki of 0.6 nM, it

elicited only modest changes in 5-HTP brain levels. However, this compd. did not appear as an antagonist when tested in a cyclic-AMP-based intrinsic activity assay.

242
Van Muiswinkel FL, Jongenelen CA M, Schepens HT W, Stoof JC, Drukarch B.
EFFECTS OF CHRONIC ACTIVATION OF DOPAMINE D-2 RECEPTORS IN CULTURES OF RAT
FETAL DOPAMINERGIC NEURONS: INDICATIONS FOR ALTERATIONS IN FUNCTIONAL ACTIVITY. Dev Brain Res 1995;85(1):128-36.

In Parkinsonian patients, previously subjected to neuronal grafting therapy, the survival and functional status of dopaminergic grafts might be impaired by the concurrent pharmacotherapy with L-DOPA and/or dopamine (DA) D-2 receptor agonists. To test this hypothesis in vitro, we studied the effects of chronic DA D-2 receptor activation on the functional capacity of cultured fetal rat mesencephalic DA neurons, using the activity of tyrosine hydroxylase (TH) and the intracellular dopamine content as neurochem. parameters. In cellular exts. prepd. from our cultures, TH activity (as detd. by the release of 3H2O from 3H-[3,5] tyrosine) appeared to be tetrahydrobiopterin-, Fe2+, and temp. sensitive, while in intact cells, the catalytic activity of TH could be induced by K+-evoked depolarization in a Ca2+-dependent way. In contrast, no acute DA D-2 receptor mediated inhibitory effects could be demonstrated in intact cells, either when tested under basal or depolarizing conditions. Nevertheless, after chronic exposure to DA D-2 receptor agonists for 14 days clear differences were obsd. in the functional status of cultured fetal dopaminergic neurons. Thus, whereas the overall survival and basal TH activity of cultured fetal dopaminergic neurons remained virtually unaltered, the depolarization induced activation of TH was enhanced in agonist-treated cultures. Moreover, after long-term treatment for 14 or 21 consecutive days, the intracellular DA content of agonist treated cultures appeared to be higher, as compared to untreated controls. It is concluded that chronic activation of DA D-2 receptors may induce adaptive alterations in the functional activity of cultured fetal dopaminergic neurons. The possible consequences of these changes for the functional activity of (grafted) fetal dopaminergic neurons are discussed.

243
Zhang Z, Wu D. [EFFECT OF METHYLMERCURY CHLORIDE ON BRAIN MUSCARINIC RECEPTOR
IN RAT.] Hua Hsi I Ko Ta Hsueh Hsueh Pao 1994;25(4):388-92. (Chi)

In this study, we found that methylmercury chloride inhibited the binding of 3H-QNB to muscarinic receptor of rat brain tissue in vitro with IC50 values of 0.0137 +/- 0.0037mol/L. It also decreased the densities of muscarinic receptor and affinity to 3H-QNB. In vivo test system, the rats were exposed to methylmercury chloride (ip) 0, 1.5, 2.5, 3.5 mg/kg.d on the 7-12th day of gestation. On the 7, 14 and 21st day after birth, the offsprings were killed, and the cerebrum and cerebellum were immediately dissected on ice and homogenizated. The ligand assays were carried out. The result showed the effect of inhibition on the binding to cerebrum and cerebellum with

significant correlation.

244
Heaton MB, Paiva M, Swanson DJ, Walker DW. ALTERATIONS IN RESPONSIVENESS TO ETHANOL AND NEUROTROPHIC SUBSTANCES IN FETAL SEPTOHIPPOCAMPAL NEURONS FOLLOWING CHRONIC PRENATAL ETHANOL EXPOSURE. Devel Brain Research 1995;85(1):1-13.

Pregnant Long-Evans rats were maintained on three diets: a liquid diet in which ethanol accounted for 35-39% of the total calories, a similar diet with the isocaloric substitution of sucrose for ethanol, and a lab chow control diet. At gestation day 18, the fetuses were taken and cultures of septal and hippocampal neurons prepared. Neuronal survival and neurite outgrowth were compared in cultures from the three diet groups, using the following media supplements: ethanol (1.2, 1.8 or 2.4 g/dl), neurotrophic factors (nerve growth factor (NGF) with the septal cultures, basic fibroblast growth factor (bFGF) with the hippocampal cultures), or ethanol plus neurotrophic factors. Both the septal and hippocampal neurons responded to ethanol in a dose-dependent manner. The neurons from both populations from fetuses which had been exposed prenatally to ethanol, however, tolerated considerably higher ethanol concentrations before decreases in survival or outgrowth were seen. These ethanol-exposed neuronal populations were also less responsive to neurotrophic factors: in hippocampal cultures, process outgrowth was significantly enhanced by bFGF in control but not ethanol-derived cultures, and in septal and hippocampal cultures, the neurotrophic factors significantly ameliorated ethanol neurotoxicity in control cultures, but not in those from the ethanol-exposed fetuses. The possible relevance of these observations to the fetal alcohol syndrome is discussed.

245
Abdulla EM, Calaminici M, Campbell IC. COMPARISON OF NEURITE OUTGROWTH WITH NEUROFILAMENT PROTEIN SUBUNIT LEVELS IN NEUROBLASTOMA CELLS FOLLOWING MERCURIC
OXIDE EXPOSURE.
Clin Exper Pharmacol Physiol 1995;22(5):362-3.

1. The objectives of the study were to establish that inhibition of neuronal differentiation in culture (assessed by neurite outgrowth) can be used as a broad spectrum in vitro measure of neurotoxicity. 2. To establish whether a rapid measure of neurite outgrowth could be used. Thus the study examined the relationship between the degree of neurite outgrowth assessed directly by image analysis and neurofilament protein subunit levels measured by an ELISA. 3. SKNSH neuroblastoma cells, exposed for up to 6 days to mercuric chloride during initiation and continuation of differentiation, had lower levels of neurofilament proteins than unexposed cells. 4. Preliminary data from parallel examinations of neurite outgrowth assessed by image analysis and neurofilament protein subunit levels assessed by ELISA support a correlation when neurofilament protein levels are decreased by sub-cytotoxic doses of mercuric chloride in SKNSH cells.

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Monnet-Tschudi F, Zurich MG, Riederer BM, Honegger P. EFFECTS OF TRIMETHYLTIN (TMT) ON GLIAL AND NEURONAL CELLS IN AGGREGATE CULTURES: DEPENDENCE ON THE
DEVELOPMENTAL STAGE. Neurotoxicology 1995;16(1):97-104.

Long term effects of trimethyltin (TMT) applied at concentrations below the cytotoxic level were examined in three-dimensional cell cultures of fetal rat telencephalon using biochemical, immunochemical and morphological criteria. It was found that in immature cultures low concentrations of TMT (10-8 M) specifically induced a gliotic response in astrocytes, with increased immunoreactivity for glial fibrillary acidic protein, and a greater number of astrocytic processes. Significant changes in oligodendrocytic and neuronal parameters were found only at 10-6 of TMT. In differentiated cultures, distinct changes in cell type-specific parameters occurred at 10-6 of TMT (the lowest effective concentration). In addition, different patterns of responses were found for astrocytes and oligodendrocytes, as compared to immature cultures. These results suggest that among neural cells, astroblasts are most sensitive to TMT, and that the glial responses to this neurotoxicant are development-dependent.

247
Chute SK, Flint OP, Durham SK. ANALYSIS OF THE STEADY-STATE DYNAMICS OF ORGANELLE MOTION IN CULTURED NEURITES: PUTATIVE INDICATOR OF NEUROTOXIC EFFECT. Clin Exp Pharmacol Physiol 1995;22(5):360-1.

The objective of this study was to develop a physiol. based method to evaluate the neurotoxic potential of drug candidates in vitro. Rat embryo midbrain cells were grown in micromass culture, and the movement of mitochondria labeled with the fluorescent dye rhodamine 123 was quantified in fasciculated neurites by using a laser cytometer. The rhodamine 123 signal in a defined region of the fascicle was quantified and photobleached with the laser. A series of post-photobleach scans revealed the movement of fluorescent-labeled mitochondria into the bleached region from adjacent unbleached regions. Recovery of fluorescence is a measure of the size of the mobile pool of mitochondria relative to the total (moving plus stationary) pool. The steady-state levels of fluorescence recovery were dependent on intracellular Ca2+ and Mg2+ concns., energy status (ATP), and microtubule integrity (post-taxol or post-vinblastine treatment). This technique may be a useful

indicator of neurotoxic effect.

248
Mueller U, Krieglstein J. PROLONGED PRETREATMENT WITH ALPHA-LIPOIC ACID PROTECTS CULTURED NEURONS AGAINST HYPOXIC, GLUTAMATE-, OR IRON-INDUCED INJURY.
J Cereb Blood Flow Metab 1995;15(4):624-30.

The antioxidant dihydrolipoic acid has been shown to reduce hypoxic and excitotoxic neuronal damage in vitro. The present study tested whether pretreatment with alpha-lipoic acid, which presumably allows endogenous formation of dihydrolipoic acid, can protect cultured neurons against injury

caused by CN-, glutamate, or Fe ions, using the trypan blue exclusion method to det. neuronal damage. One hour of preincubation with dihydrolipoic acid (1 muM), but not with alpha-lipoic acid, reduced damage to neurons from chick embryo telencephalon caused by 1 mM NaCN or Fe ions. Alpha-Lipoic acid (1 muM) reduced CN--induced neuronal damage when added 24 h before hypoxia, and pretreatment with alpha-lipoic acid for >24 h enhanced this neuroprotective effect. Both the R- and the S-enantiomers of alpha-lipoic acid exerted a similar neuroprotective effect. Pretreatment with alpha-lipoic acid (1 muM) from the day of plating onward prevented the degeneration of chick embryo telencephalic neurons that had been exposed to Fe2+/Fe3+. Alpha-Lipoic acid (1 muM) added to the culture medium the day of plating also reduced neuronal injury induced by 1 mM L-glutamate in rat hippocampal cultures, whereas 30 min of preincubation with alpha-lipoic acid failed to attenuate glutamate-induced neuronal damage. The results indicate that neuroprotection by prolonged pretreatment with alpha-lipoic acid is probably due to the radical-scavenger properties of endogenously formed dihydrolipoic acid.

249
Sawyer T. PRACTICAL APPLICATIONS OF NEURONAL TISSUE CULTURE IN IN VITRO TOXICOLOGY. Clin Exp Pharmacol Physiol 1995;22(4):295-6.

1. Primary chick embryo forebrain neurones are relatively easy to culture and are quite resilient to treatment manipulation. These characteristics have allowed application in a surprisingly diverse number of areas. 2. These cultures have been used to investigate the anticholinesterase potencies of many organophosphate (OP) nerve agents and insecticides. 3. These cultures have been used to quantitate levels of OP in 'spiked' unknowns and in OP-contaminated soil samples. 4. These cells have also been used to test a variety of compounds using the MTT and neutral red cytotoxicity assays.

250
Freeman JK, Goldberg MP. CONFOCAL MICROSCOPIC VISUALIZATION OF MK-801-INDUCED CYTOPLASMIC VACUOLES IN VITRO. Psychopharmacol Bull 1994; 30(4):541-7.

We examined neuroprotective and neurotoxic effects of the NMDA antagonist, MK-801, in primary cell cultures derived from embryonic mouse neocortex. Brief deprivation of oxygen and glucose, or direct application of N-methyl-D-aspartate (NMDA), resulted in acute neuronal swelling followed by neuronal death during the next day. This excitotoxic neuronal injury could be blocked by inclusion of a wide variety of NMDA antagonists in the cell culture medium. MK-801 attenuated neuronal death in the low micromolar range; 1 to 10 microM concentrations were sufficient to maximally reduce injury from NMDA toxicity, oxygen deprivation, or combined deprivation of oxygen and glucose. MK-801 alone caused no apparent toxicity at these concentrations in exposures of 24 to 48 hours. However, 24-hour exposure to 100 microM MK-801 resulted in appearance of cytoplasmic vacuoles, which could be visualized with immunofluorescence against the microtubule associated protein, MAP2, together with laser scanning confocal microscopy. Thus, at concentrations sufficient to block NMDA receptors, MK-801 is neuroprotective rather than neurotoxic for cortical neurons in vitro. This model system may provide a method to examine cellular mechanisms underlying the neurotoxicity of MK-801 at very high

concentrations.

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Abdulla EM, Atterwill C, Campbell IC. WORKSHOP ON IN VITRO NEUROTOXICITY TESTING THE OBSTACLES. THE WAY FORWARD.
VAL MORIN CANADA JULY 1994. Clin Exp Pharmacol Physiol 1995;22(4):277-80.

252
Vaalavirta L, Tahti H. EFFECTS OF SELECTED ORGANIC SOLVENTS ON THE ASTROCYTE MEMBRANE ATPase IN VITRO. Clin Exp Pharmacol Physiol 1995;22(4):293-4.

1. The present study deals with astrocyte cultures as a model for studying the membrane-mediated central nervous system-depressing effect of organic solvents. 2. The primary astrocyte cultures were prepared from neonatal rat cerebella. The cells were cultured in modified essential medium. The astrocyte membranes isolated from the cultures were exposed to solvents in incubation mixture at different dose levels (3, 6 and 9 mmol/L) for 1 h. The physiologically important integral proteins Na+, K+-ATPase and Mg2+-ATPase were studied. 3. The aromatic hydrocarbons (benzene, toluene, styrene, xylene and ethylbenzene) inhibited the ATPase activities according to their lipid solubilities. n-Hexane and cyclohexane clearly had less effect than aromatic hydrocarbons, despite their greater lipid solubilities. 4. Astrocytes were shown to be sensitive targets to the effects of organic solvents, measured as the inhibition of the integral enzymes Na+, K+-ATPase and Mg2+-ATPase.

253
Martenson CH, Sheetz MP, Graham DG. IN VITRO ACRYLAMIDE EXPOSURE ALTERS GROWTH
CONE MORPHOLOGY. Toxicol Appl Pharmacol 1995;31(1):119-29.

Acrylamide intoxication leads to degeneration of the longest axons of the central and peripheral nervous systems in humans and laboratory animals. Axonal derangements resulting from in vivo acrylamide exposure are first noted within synapses of the longest axons before involving more proximally located axonal segments or shorter axons, thus illustrating the specificity of acrylamide for the terminal axonal regions. As a possible model system for investigating the mechanism of toxicity of acrylamide on the distal axon, we exposed neurite-extending chick dorsal root ganglion (DRG) cells to acrylamide in vitro and then examined growth cones for alterations in morphology and function. Exposing DRG explants to media containing from 0.125 to 1.0 mM acrylamide for 16 hr leads to specific and dose-responsive alterations of growth cone morphology including: a nearly total loss of filopodial elements, the preservation of highly active but two-dimensional lamellar structures, an inappropriate extension of the axonal cytoskeleton into the forward region of most growth cones, and a frequent breakdown of the central and peripheral growth cone domains. The sulfhydryl alkylating agents ethacrynic acid, iodoacetamide, and iodoacetic acid were tested and none produced acrylamide-like morphological alterations at any dose. DRG cultures were also exposed to the neurotoxic acrylamide analogs glycidamide, N-hydroxy methacrylamide (HM-ACR), and methacrylamide (M-ACR). At concentrations of 0.25 to 1.0 mM, glycidamide exposure resulted in acrylamide-like growth cone

alterations. HM-ACR exposure also resulted in growth cones that were acrylamide-like but only at concentrations > 1.5 mM. M-ACR did not produce acrylamide-like growth cones at doses of up to 16.6 mM. Thus, in vitro exposure of DRG explants to acrylamide and two neurotoxic acrylamide analogs leads to reproducible and specific morphological alterations that are dose-dependent and separable from the effects of sulfhydryl alkylation.

254
Ehrich M. USING NEUROBLASTOMA CELL LINES TO ADDRESS DIFFERENTIAL SPECIFICITY TO ORGANOPHOSPHATES. Clin Exp Pharmacol Physiol 1995; 22(4):291-2.

1. Organophosphates can cause acute toxicity, which follows inhibition of acetylcholinesterase (AChE), or delayed neuropathy, which follows inhibition of neuropathy target esterase (NTE). 2. Human neuroblastoma SH-SY5Y cells contain AChE and NTE. 3. Organophosphates actively able to inhibit AChE in animal models inhibited AChE in neuroblastoma cells. 4. Inhibition of NTE in neuroblastoma cells could identify active organophosphates capable of causing delayed neuropathy in animal models and distinguish these organophosphates from those that do not cause delayed neuropathy in animal models.

255
Durham HD, O'Brien C, Nalbantogul J, Figlewicz DA. USE OF TISSUE CULTURE MODELS TO STUDY ENVIRONMENTAL-GENETIC INTERACTIONS RELEVANT TO NEURODEGENERATIVE DISEASES. Clin Exp Pharmacol Physiol 1995;22(5): 366-7.

1. Clonal cell lines, primary cultured neurones and transgenic animals expressing mutant genes linked to familial forms of neurodegenerative diseases provide models in which to examine the interaction between expression of a predisposing gene and exposure to neurotoxic chemicals. Methods of establishing these models are reviewed. 2. Mutations in the gene encoding Cu/Zn-superoxide dismutase (SOD-1) have been identified in cases of familial amyotrophic lateral sclerosis linked to chromosome 21. We report that in clonal lines of PC12 cells, the cytotoxicity of a glutathione-depleting epoxide, styrene oxide, varied with SOD activity in a manner similar to that previously demonstrated for redox cycling chemicals. These preliminary data suggest that either low or high SOD-1 activities may be associated with greater toxicity of a variety of neurotoxic chemicals and their metabolites.

256
Carbonnelle P, Lison D, Leroy JY, Laurerys R. EFFECT OF THE BENZENE METABOLITE HYDROQUINONE, ON INTERLEUKIN-1 SECRETION BY HUMAN MONOCYTES IN VITRO. Toxicol
Appl Pharmacol 1995;132(2):220-6.

Decreased interleukin-1 (IL-1) production by mononuclear phagocytes has been shown to contribute to benzene myelotoxicity in animals. The study presented here was designed to examine the relevance of this mechanism in humans. Fresh human blood monocytes were exposed to 0.001-10 muM hydroquinone (HQ) and assessed for their ability to release IL-1alpha and IL-1beta in response to a stimulation with endotoxin. Both cytokines were measured by specific ELISA.

Exposure of human monocytes to micromolar concentrations of HQ for 2 hr resulted in a dose-dependent reduction of IL-1 secretion. For both IL-1alpha and IL-1beta, the decreases were statistically significant at concentrations of 5 muM and above. HQ also inhibited RNA and protein synthesis in a dose-dependent manner, with 50% inhibitory concentrations of 21 : 11 and 10 : 9 muM, respectively. Furthermore, monocytes treated with 5 muM HQ also displayed a reduced total protein content when compared with control cells. These data suggest tha the reduction of IL-1 production caused by HQ results from a global impairment of monocyte essential functions such as transcription or translation. Taken as a whole, our results support a mechanism whereby HQ may contribute to the myelotoxicity of benzene in humans by inhibiting the production by mononuclear phagocytes of cytokines involved in the regulation of hematopoiesis.

257
Kohila T, Hypponen S, Tahti H. THE NEUROTOXICITY OF LEAD STUDIED WITH RAT SYNAPTOSOMAL INTEGRAL PROTEINS ATPase AND ACETYL-CHOLINESTERASE. Neurosci Res
Commun 1995;16(3):173-80.

The effects of inorganic lead on synaptosomal cell membrane integral proteins were studied both in vivo and in vitro. In in vivo studies, outbred WI:Han rats from Laboratory Animal Centre, Helsinki University were exposed to inorganic lead ($PbCO_3$), 29.0 mg/kg in the diet ad libitum. In the control diet the amount of lead was 0.58 mg/kg. The experimental animals were exposed to lead as follows: the mothers were eating the test diet during the whole pregnancy and the suckling. After weaning the pups were fed with the test diet for two months. This exposure caused a slight increase of blood lead level (B-Pb 0.35 muM/l) compared to controls (B-Pb 0.20 muM/l). In the in vitro study, incubation mixtures had known lead concentrations of 3, 6, 9, 15 and 30 muM/l. Synaptosomal membranes were isolated from rat cerebrum by using a nontoxic isoosmotic Percoll gradient system. The integral proteins studied were ATPase and acetylcholinesterase (AChE), both important in the normal functioning of synaptosomal membranes. Inorganic lead in vitro caused a marked dose-dependent decrease in Na^+,K^+-ATPase activity, but not in Mg^{2+}-ATPase and in AChE activity. After in vivo exposure, there was a slight but statistically not significant decrease in the enzyme activities studied.

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Kerr JE, Allore RJ, Beck SG, Handa RJ. DISTRIIBUTION AND HORMONAL REGULATION OF ANDROGEN RECEPTOR (AR) AND AR MESSENGER RIBONUCLEIC ACID IN THE RAT HIPPOCAMPUS. Endocrinology 1995; 136(8):3213-21.

The actions of androgens in both peripheral and central tissues are linked in part to their ability to specifically bind and activate androgen receptors (ARs). ARs have been well studied in the rat hypothalamus and peripheral reproductive tissues, where they are directly involved in endocrine feedback mechanisms and reprodn. Previous studies revealed relatively high levels of AR and AR mRNA in the rat hippocampus; however, the action of androgen in this brain region remains unclear. To begin to address this issue, we used a multidisciplinary approach to quantitate hippocampal AR and AR mRNA levels and

investigate their regulation after various hormonal manipulations. In vitro binding assays revealed a single, saturable, high affinity binding site for androgen in hippocampal cytosols. The expression of AR mRNA in the intact adult male rat hypothalamus and hippocampus was demonstrated using reverse transcription-polymerase chain reaction and quantified using a RNase protection assay. Comparable levels of AR mRNA were found in the hippocampus and hypothalamus. In addn., in situ hybridization anal. revealed a unique distribution of AR mRNA in the hippocampus. AR mRNA was found predominately in the CA1 pyramidal cells, which form the major signal output of the hippocampal trisynaptic circuit. Reverse transcription-polymerase chain reaction of total RNA from microdissected hippocampal regions confirmed this distribution. RNase protection assay demonstrated a significant decrease in the AR mRNA content of the hippocampus in animals killed 4 days after castration or in intact rats after four daily injections of the AR antagonist, flutamide (15 mg/animal), compared to that in intact controls. In contrast, a 35% increase in the hippocampal AR mRNA content was found in old (22-mo-old) compared to young (5-mo-old) male rats. In both cases, [3H]dihydrotestosterone binding to the cytosolic prepn. did not parallel the changes obsd. in the AR mRNA content. Taken together, these data demonstrate that hippocampal cells contg. AR can respond to circulating androgen to alter AR gene expression. Furthermore, AR mRNA autoregulation appears to be both age and tissue specific and does not directly follow the regulatory patterns described for other steroid hormone receptors found in the hippocampus.

259
Skofitsch G. LIGHT MICROSCOPIC IN VITRO RECEPTOR AUTORADIOGRAPHY: X-RAY FILM VISUALIZATION OF NEUROPEPTIDE RECEPTOR BINDING SITES IN RAT BRAIN. Mod Methods Anal Morphol [Proc. Int. Workshop Mod. Anal. Methods Histochem.] 1994;361-79.

A general and simple autoradiog. procedure to visualize neuropeptide receptor binding sites in rat brain tissue sections is described in detail. The method utilizes unfixed slide-mounted cryostat tissue sections of the rat brain which are incubated with 125I labeled neuropeptides. Visualization of binding sites is achieved by direct exposure of radiolabeled tissue sections to x-ray film in autoradiog. cassettes. For example, binding of 125I-porcine galanin to coronal sections of the rat brain is shown. Details of the autoradiog. procedure are discussed.

260
Zhou A, Guo J, Wang H, Gu B, Du Y. ENHANCEMENT OF NGF GENE EXPRESSION IN RAT BRAIN BY THE MEMORY-ENHANCING PEPTIDE AVP(4-8). Peptides 1995;16(4):581-6.

Northern blot anal. of nerve growth factor (NGF) was used to evaluate the effect of exogenous AVP(4-8) on the transcription of NGF gene in rat brain. NGF expression was significantly enhanced by exogenous AVP(4-8) in the hippocampus as well as in the cerebral cortex in a time period of 12 h. This effect was inhibited by an antagonist to AVP(4-8). In addn., gel mobility shift assay was also used to observe the in vitro expression of c-fos gene in

rat hippocampal slices. The results suggest that NGF gene is one of the target genes responsible for memory-enhancing responses induced by AVP(4-8) and that the enhancement of NGF gene expression may share the signaling pathway mediated by AVP(4-8) receptor and c-fos gene expression.

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Li J, Bigge CF, Williamson RM, Borosky SA, Vartanian MG, Ortwine DF. POTENT, ORALLY ACTIVE, COMPETITIVE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR ANTAGONISTS
ARE SUBSTRATES FOR A NEUTRAL AMINO ACID UPTAKE SYSTEM IN CHINESE HAMSTER OVARY
CELLS. J Med Chem 1995; 38(11):955-65.

A series of enantiomerically pure (phosphonomethyl)-substituted phenylalanine derivs. related to SDZ EAB 515 (I) were prepd. as competitive N-methyl-D-aspartate (NMDA) receptor antagonists. Unlike most known competitive NMDA antagonists, analogs in this series with the S-configuration are potent NMDA antagonists whereas analogs with the unnatural R-configuration are weak NMDA antagonists, as detd. by receptor binding expts. and their anticonvulsant action in mice. Examn. in a previously reported competitive NMDA pharmacophore model revealed that receptor affinity can be explained partially by a cavity that accommodates the biphenyl ring of I, while the biphenyl ring of the R-enantiomer extends into a disallowed steric region. We proposed that analogs with the natural S-configuration and a large hydrophobic moiety would have an advantage in vivo over analogs with an R-configuration by being able to use a neutral amino acid uptake system to enhance both peripheral adsorption and transport into the brain. Examn. in a system L neutral amino acid transport carrier assay shows that 1 competes with L-Phe for transport in an apparent competitive and stereospecific manner (estd. Ki = 50 .mu.M). The 1- and 2-naphthyl derivs. (II and III) were among the most potent, competitive NMDA antagonists yet discovered, being ca. 15-fold more

potent than I in vitro and in vivo, with a long duration of action. The title compd. II had potent oral activity in MES (ED50 = 5.0 mg/kg). II also retains its ability to compete, albeit more weakly than I (estd. Ki = 200 muM), for L-Phe uptake to CHO cells. In this series, analogs with the R-configuration are not substrates for the system L neutral amino acid transport carrier. These results provide evidence that central nervous system active agents can be designed as substrates of a neutral amino acid transporter as a means to enhance penetration of the blood-brain barrier.

262
Dicicco-Bloom E, Black IB. BLOOD-BRAIN BARRIER TRANSPORTERS OF NEUROLOGICAL AGENTS DERIVED FROM GROWTH FACTORS. PCT Int. Appl. PATENT NO. 95 07092 03/16/95 (University of Medicine and Dentistry of New Jersey).

A method for inducing neuronal precursor cells (NPCs) in vitro and in vivo using proliferation factors to promote mitosis, survival or differentiation of at least one type of NPC in the treatment of neuronal cell disorders caused by disease, injury, and other neural disorders is described. Methods for transporting mols. across the blood brain barrier by linking them to fragments

of growth factors to effectively introduce neurol. agents capable or incapable of independently crossing the blood brain barrier into the central nervous system is a major component of the treatment. Such substances include peptides having the amino acid sequence Val Phe Phe. The in vivo and in vitro regulation of cerebellar granule cell neurogenesis by basic fibroblast growth factor is demonstrated.

263
Prasad JA, Shukla VK, Lemaire S. SYNTHESIS AND BIOLOGICAL ACTIVITY OF HISTOGRANIN AND RELATED PEPTIDES. Can J Physiol Pharmacol 1995; 73(2):209-14.

Histogranin (HN) was first isolated from bovine adrenal medulla and shown to be a pentadecapeptide displaying N-methyl-D-aspartate (NMDA) receptor antagonist activity. To det. the active pharmacophore of HN, fragments of the peptide were synthesized and their structure-activity relationships studied by measuring their ability to displace the binding of [125I][Ser1]HN to rat brain membrane prepns. and to block NMDA-induced convulsions in mice. In the binding assay, only the full length peptide HN(1-10) displayed a high affinity (Ki of 72 and 162 nM, resp.). All other tested fragments with deletions at the N- and(or) C-terminals of the mol. showed large (16-2500-fold) decreases in potency. The least active peptide fragment tested was HN(6-10) (Ki of 164 muM). In vivo, HN and HN(2-15) (100 nmol; i.c.v.) produced 94 and 40% protection against NMDA-induced convulsions in mice, resp. None of the other peptide fragments displayed significant anticonvulsant activity. The protective activity of HN (60 and 100 nmol) was markedly antagonized by coadministration of HN(1-10) (100 nmol). The results indicate that the in vivo anti-NMDA and in vitro binding activities of HN and related peptides, with the exception of HN(1-10), depend upon the integrity of the mol. The high affinity of HN(1-10) for HN binding sites correlates well with its antagonist effects towards the activity of the parent peptide.

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Kiso K, Watanabe Y, Shibuya T. CENTRAL MECHANISM OF A NOVEL NEUROTOXIC, PARAQUAT, AND ITS RELATIONSHIP TO INCREASED AMOUNTS OF EXCITATORY AMINO ACIDS.
Neurosciences 1994;20(4):169-79.

The neurotoxic mechanism of paraquat (PQ; 1,1'-dimethyl-4,4'-bipyridylium) in the central nervous system due to the excessive release of brain excitatory amino acids was studied using glia-rich and -poor cell cultures derived from neonatal rat cerebellar granule cells. In glia-poor cell cultures, a 15-min exposure to PQ (2 mM, 37.degree.) significantly enhanced the 50 mM KCl-evoked glutamate (Glu) and taurine (Tau) release, but not glutamine (Gln) and glycine (Gly) release. In glia-rich cell cultures, PQ tended to amplify the high potassium-evoked release of the 4 amino acids, but these increases were not significant. The enhancement of Glu release by PQ in the glia-poor cell cultures was much greater than that in the glia-rich cell cultures. These differences might be explained by the uptake of much of the released Glu by the glial cells, because the potentiation of Gln synthetase activity was higher in glia-rich cell cultures than in glia-poor cell cultures after the treatment with PQ. Neuronal cell function in glia-poor cell cultures was

markedly damaged after a 30-min exposure to PQ (2 mM, 37.degree.) using the lactate dehydrogenase (LHD) assay. Furthermore, in previously reported in vitro studies, PQ increased the sustained rise of Ca2+ levels in the rat brain synaptosomal fraction, and such Ca2+ increase was remarkably potentiated by 50 mM KCl (Pharmacol. and Toxicol., 1993). The above results suggest that part of the PQ-induced neurotoxicity is assocd. with the increased release of Glu caused by the unstable depolarizing condition of brain neuronal cells.

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Woodward RM, Huettner JE, Guastella J, Keana JF, Weber E. IN VITRO PHARMACOLOGY OF ACEA-1021 AND ACEA-1031: SYSTEMICALLY ACTIVE QUINOXALINEDIONES
WITH HIGH AFFINITY AND SELECTIVITY FOR N-METHYL-D-ASPARTATE RECEPTOR GLYCINE
SITES. Mol Pharmacol 1995; 47(3):568-81.

N-methyl-D-aspartate (NMDA) receptor antagonists show therapeutic potential as neuroprotectants, analgesics, and anticonvulsants. In this context, we used elec. recording techniques to study the in vitro pharmacol. of two novel quinoxalinediones, i.e., ACEA-1021 and ACEA-1031 (5-nitro-6,7-dichloro- and 5-nitro-6,7-dibromo-1,4-dihydro-2,3-quinoxalinedione, resp.). Assays with NMDA receptors expressed by rat brain poly(A)+ RNA in Xenopus oocytes and with NMDA receptors in cultured rat cortical neurons indicated that ACEA-1021 and ACEA-1031 are potent competitive antagonists at NMDA receptor glycine sites. Apparent dissocn. consts. (Kb values) for ACEA-1021 and ACEA-1031 ranged between 6 and 8 nm for oocyte assays and between 5 and 7 nM for neuronal assays. Cloned NMDA receptors expressed in oocytes showed up to 50-fold variation in sensitivity, depending upon subunit compn. For example, using fixed agonist concns. (10 muM glycine and 100 muM glutamate) IC50 values for ACEA-1021 with four binary combinations were as follows: NMDA receptor (NR)1A/2A, 29 nM; NR1A/2B, 300 nM; NR1A/2C, 120 nM; NR1A/2D, 1500 nM. Measurement of EC50 for glycine and calcn. of Kb for the inhibitors indicated that differences in IC50 values are due to subunit-dependent variations in glycine affinity (EC50 ranged between .apprx.0.1 and 1 muM) combined with variations in affinity of the antagonists themselves (Kb of .apprx.2-13 nM). In addn. to the strong antagonism of NMDA receptors, ACEA-1021 and ACEA-1031 were also moderately potent competitive inhibitors of non-NMDA receptors activated either by alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid or by kainate. Antagonist affinities were similar whether measured with receptors expressed by rat brain poly(A)+ RNA in oocytes (Kb of 1-2 muM) or with cultured neurons (Kb of 1.5-3.3 .mu.M). Our results suggest that the in vivo neuroprotective actions of ACEA-1021 and ACEA-1031 are predominantly due to inhibition at NMDA receptor glycine sites, although addnl. inhibition at non-NMDA receptors may play an ancillary role.

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Bilsky EJ, Calderon SN, Wang T, Bernstein RN, Davis P, Hruby VJ, McNutt RW, Rothman RB, Rice KC, Porreca F. SNC 80, A SELECTIVE, NONPEPTIDIC AND SYSTEMICALLY ACTIVE OPIOID DELTA AGONIST. J Pharmacol Exp Ther 1995;273(1):359-66.

The present study has investigated the pharmacol. of SNC 80, a nonpeptidic ligand proposed to be a selective delta agonist in vitro and in vivo. SNC 80 was potent in producing inhibition of elec. induced contractions of mouse vas deferens, but not in inhibiting contractions of the guinea pig isolated ileum (IC50 values of 2.73 nM and 5457 nM, resp.). The delta selective antagonist ICl 174,864 (1 muM) and the mu selective antagonist CTAP (1 muM) produced 236- and 1.9-fold increases, resp., in the SNC 80 IC50 value in the mouse vas deferens. SNC 80 preferentially competed against sites labeled by [3H]naltrindole (delta receptors) rather than against those labeled by [3H]DAMGO (mu receptors) or [3H]U69, 593 (kappa receptors) in mouse whole-brain assays. The ratios of the calcd. Ki values for SNC 80 at mu/delta and kappa/delta sites were 495- and 248-fold, resp., which indicates a significant degree of delta selectivity for this compd. in radioligand binding assays. SNC 80 produced dose- and time-related antinociception in the mouse warm-water tail-flick test after i.c.v., i.th. and i.p. administration. The calcd. A50 values (and 95% C.I.) for SNC 80 administered i.c.v., i.th. and i.p. were 104.9 (63.7-172.7) nmol, 69 (51.8-92.1) nmol and 57 (44.5-73.1) mg/kg, resp. The i.c.v. administration of SNC 80 also produced dose- and time-related antinociception in the hot-plate test, with a calcd. A50 value (and 95% C.I.) of 91.9 (60.3-140.0) nmol. I.p. SNC 80 antinociception was antagonized by pretreatment with i.c.v. naloxone (3 nmol), with i.c.v. or i.th. N,N-diallyl-Tyr-(Aib)2-Phe-Leu-OH(Aib=alpha-amino isobutyric acid) (4.4 nmol) or with i.p. naltrindole (20 mg/kg), but not i.c.v. or i.th. beta-FNA (18.8 nmol at -24 h). Furthermore, the antinociceptive effects of i.c.v. SNC 80 were antagonized by i.c.v. pretreatment with either [D-Ala2,Leu5,Cys6]enkephalin (a putative delta1 antagonist) or [D-Ala2, Cys4]deltorphin (a putative delta2 antagonist), but not by beta-funaltrexamine (a mu antagonist). This suggests that the antinociceptive actions of SNC 80 are produced via both opioid delta1 and delta2, but not mu, receptors. On the basis of its profile in vivo and in vitro, SNC 80 is perhaps the first highly selective, nonpeptidic and systemically active opioid delta agonist. SNC 80 promises to be a useful compd. for the exploration of opioid delta-receptor pharmacol. and provides a basis for the further identification of selective nonpeptidic delta ligands.

267
Greer JJ, Carter JE. EFFECTS OF CYANIDE ON THE NEURAL MECHANISMS CONTROLLING BREATHING IN THE NEONATAL RAT IN VITRO. Neurotoxicology 1995;16(2):211-5.

The effects of hydrogen cyanide (HCN) on the neutral mechanisms controlling breathing were studied. Two in vitro experimental models were utilized: the brain stem-spinal cord and the medullary slice preparations isolated from neonatal rats. Cyanide, at concentrations deemed lethal in vivo (50 muM), caused a modest (< 15%) depression of the frequency and amplitude of inspiratory rhythmic discharge when added to the bathing media. Moreover, the neuronal network underlying respiratory rhythmogenesis continued to function for hours in the presence of very high concentrations of cyanide (600 muM). We hypothesize that the rapid suppression of breathing caused by cyanide in vivo is due to changes in neuronal excitability in respiratory modulating populations in the CNS rather than due to perturbations of cellular oxidative metabolism of neurons within respiratory rhythm generating centres.

268
Deshpande SS, Smith CD, Filbert MG. ASSESSMENT OF PRIMARY NEURONAL CULTURE AS A MODEL FOR SOMAN-INDUCED NEUROTOXICITY AND EFFECTIVENESS OF MEMANTINE AS A
NEUROPROTECTIVE DRUG. Arch Toxicol 1995;69(6):384-90.

An in vitro mammalian model neuronal system to evaluate the intrinsic toxicity of soman and other neurotoxicants as well as the efficacy of potential countermeasures was investigated. The link between soman toxicity, glutamate hyperactivity and neuronal death in the central nervous system was investigated in primary dissociated cell cultures from rat hippocampus and cerebral neocortex. Exposure of cortical or hippocampal neurons to glutamate for 30 min produced neuronal death in almost 80% of the cells examined at 24 h. Hippocampal neurons exposed to soman for 15-120 min at 0.1 mum concentration caused almost complete inhibition (> 90%) of acetylcholinesterase but failed to show any evidence of effects on cell viability, indicating a lack of direct cytotoxicity by this agent. Acetylcholine (ACh, 0.1 mM), alone or in combination with soman, did not potentiate glutamate toxicity in hippocampal neurons. Memantine, a drug used for the therapy of Parkinson's disease, spasticity and other brain disorders, significantly protected hippocampal and cortical neurons in culture against glutamate and N-methyl-D-aspartate (NMDA) excitotoxicity. In rats a single dose of memantine (18 mg/kg) administered 1 h prior to a s.c. injection of a 0.9 LD50 dose of soman reduced the severity of convulsions and increased survival. Survival, however, was accompanied by neuronal loss in the frontal cortex, piriform cortex and hippocampus.

269
Struzynska L, Dabrowska-Bouta B, Lenart J, Zborowska J, Rafalowska U. SOME METABOLIC EFFECTS IN RAT BRAIN SYNAPTOSOMES AFTER EXPOSURE TO LEAD IN VIVO AND
IN VITRO. Bull Pol Acad Sci Biol Sci 1994;42(1):55-62.

The mechanisms of the toxicity effect of lead on the central nervous system are not clear. Our earlier investigation on isolated synaptosomes confirmed a hypothesis, that Pb2+/Ca2+ interactions may play an important role in release of neurotransmitter from synaptosomes. However, we have been also shown, that Pb-toxicity effect on uptake of neurotransmission is not necessarily connected with Pb2+/Ca2+ interactions. Thus, we look for other mechanisms, which can be responsible for Pb-toxicity effects on neurotransmitters uptake. In the present work we demonstrated that administration of Pb(CH3COO)2 in vivo and in vitro did not affect phospholipid composition and content in synaptosomes and the lipid peroxidation, but inhibit Na+-K+-ATPase activity. The decrease of Na+-K+-ATPase activity can change the gradients of sodium and potassium across the cell membrane and can be the cause of the disturbances in neurotransmitters uptake.

270
Matsuda M, Tsukada N, Miyagi K, Yanagisa WA. ADHESION OF LYMPHOCYTES TO

ENDOTHELIAL CELLS IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS BEFORE AND AFTER
TREATMENT WITH ENDOTOXIN LIPOPOLYSACCHARIDE. Int Arch Allergy Immunol 1995;106(4):335-44.

We investigated the in vitro adhesion of 51Cr-labeled lymphocytes to cultured brain endothelial cells and the in vivo expression of intercellular adhesion molecule-1 (ICAM-1) on cerebral endothelial cells in a rat model of experimental allergic encephalomyelitis (EAE) before and after treatment with lipopolysaccharide (LPS). Adhesion of lymphocytes to cerebral endothelial cells was significantly increased in EAE compared with controls ($p < 0.01$), and was significantly correlated with the percentage of major histocompatibility complex class II antigen-positive cells in lymph node cells ($p<0.001$). LPS enhanced ICAM-1 expression on endothelial cells and lymphocyte adhesion to those cells, and caused a significant increase in the in vivo expression of ICAM-1 compared with controls ($p<0.001$). Lymphocyte adhesion to endothelial cells was significantly blocked by monoclonal antibodies against ICAM-1, lymphocyte function-associated antigen-1, or very late activation antigen-4. Our findings suggest that lymphocyte adhesion to brain endothelial cells may contribute to lymphocyte migration across the blood-brain barrier in EAE and that LPS may cause progression of EAE lesions.

271
Mundy WR, Freudenrich T, Shafer TJ, Nostrandt AC. IN VITRO ALUMINUM INHIBITION OF BRAIN PHOSPHOINOSITIDE METABOLISM: COMPARISON OF NEONATAL AND ADULT RATS.
Neurotoxicology 1995;16(1):35-44.

Recent evidence indicates that the neurotoxic metal aluminum interferes with the phosphoinositide second messenger system in adult rats both in vitro and in vivo. We have examined the age-related effects of aluminum chloride ($AlCl_3$) on receptor-stimulated inositol phosphate (IP) accumulation in brain slices from neonatal and adult rats in vitro. Carbachol-stimulated (1 mM) IP accumulation was greatest in frontal cortex slices from 7 day old rats, decreased in 14 day old and 21 day old rats, and was lowest in adults (120 days old). $AlCl_3$ (500 muM) inhibited both basal and carbachol-stimulated IP accumulation in neonatal and adult rats. The effects of $AlCl_3$ were concentration-related and produced significant decreases (15-25%) in IP accumulation at 500 and 1000 muM. The concentration-response curve for $AlCl_3$ was similar in 7 day old and adult rats. $AlCl_3$ reduced carbachol-, norepinephrine- and quisqualate-stimulated IP accumulation in both 7 day old and adult rats. The effects of 500 muM $AlCl_3$ were examined on carbachol-stimulated IP accumulation in slices prepared from frontal cortex, hippocampus, striatum, and cerebellum. Although IP accumulation was greater in slices from the 7 day old rats compared to adults in each tissue, $AlCl_3$ (500 muM) decreased IP accumulation by approximately 20% in all regions at both ages. Aluminum produced concentration-dependent inhibition of phospholipase C in cortical homogenates which was similar in 7 day old and adult rats. These results show that in vitro exposure to aluminum decreases IP accumulation through a mechanism which is not age-dependent.

272
Jett DA, Guilarte TR. DEVELOPMENTAL LEAD EXPOSURE ALTERS N-MEYTHL-D-ASPARTATE
AND MUSCARINIC CHOLINERGIC RECEPTORS IN THE RAT HIPPOCAMPUS: AN AUTORADIOGRAPHIC STUDY. Neurotoxicology 1995; 16(1):7-18.

We have used quantitative autoradiography of the N-Methyl-D-Aspartate (NMDA) receptor non-competitive antagonist (+)-5-methyl-10, 11-dihydro-5H-dibenzo(a,d)-cyclohepten-5, 10-imine maleate in the tritiated form ((3H)-MK-801) and the muscarinic cholinergic receptor antagonist (3H)-N-Methylscopolamine ((3H)-NMS) to determine the effects of developmental exposure to lead (Pb) on NMOA and muscarinic receptors in the rat hippocampus. Exposure to Pb during development resulted in age-specific changes in the level of binding of both ligands to their respective receptors. Pb exposure caused significant increases (19-49%) in (3H)-MK-801 binding throughout the hippocampus and entorhinal cortex (ECTX) of postnatal day (PN) 14 rats relative to controls. Small but significant region-specific reductions (14-18%) and increases (12-15%) in (3H)-MK-801 binding were measured in PN28 rats. No significant differences in (3H)-MK-801 binding were observed among any of the groups at PN56. Unlike (3H)-MK-801 binding, the most significant effects of Pb on (3H)-NMS binding were reductions in binding of 10-20% observed in the hippocampus and ECTX of Pb-exposed rats at PN14. Additionally, Pb in vitro had no effect on the binding of (3H)-NMS to brain sections (10 muM Pb acetate) or neuronal membrane preparations (0.1 nM to 0.1 mM Pb acetate) from normal neonatal or adult rats. These findings indicate that there may be a critical developmental window during which Pb effects are most pronounced, and the magnitude and direction of these changes is dependent upon the age of the animal and the neurotransmitter system being examined. It is suggested that NMDA and muscarinic receptors play an important role in the developmental toxicity of Pb.

273
Kisby GE, Ross SM, Spencer PS, Gold BG, Nunn PB, Roy DN. CYCASIN AND BMAA: CANDIDATE NEUROTOXINS FOR WESTERN PACIFIC AMYOTROPHIC LATERAL SCLEROSIS/PARKINSONISM-DEMENTIA COMPLEX. Neurodegeneration 1992;1(1):73-82.

Medicinal and food use of seed of the neurotoxic cycad plant (Cycas spp.) is a possible etiological factor for a model, age-linked neurodegenerative disease frequenting certain Chamorro (Guam and Rota, Mariana Islands), Japanese (Honshu island) and Papuan (west New Guinea) populations. The cycad plant toxins beta-N-methylamino-L-alanine (BMAA) and cycasin (methylazoxymethanol beta-Dglucoside) enter rodent brain tissue in vitro by a sodium-dependent mechanism (BMAA) or via a mechanism compatible with glucose transport (cycasin). The uptake of radiolabel from (3H)-BMAA into mouse brain synaptosomes is dependent on protein concentration and optimal at pH 7.4-9.0. Cycasin uptake into mouse cortical explants is concentration- and time-dependent with significant levels of the ultimate toxic metabolite methylazoxymethanol (MAM) produced 24 h after glucoside treatment. Cycasin (100 muM) significantly inhibited the uptake of (3H)-2-deoxyglucose (2-DG), a marker for glucose transport, into mouse cortical explants. Treatment of mouse cortical explants with cycasin (1.0 muM-1000 muM) induced selective

neuronal degeneration with extensive vacuolation of neuronal perikaraya, neuropil and post-synaptic elements. The uptake and subsequent intracellular actions of these two cycad chemicals are potential mechanisms by which these agents trigger their neurotoxic effects. Further research is underway to characterize the intracellular actions of BMAA and cycasin, and to determine their potential role in the prototypical western Pacific neurodegenerative disorder.

274
Gerasimyak GR, Rozanov VA, Shafran LM. [STUDY OF BIS-(N-TRIBUTYLTIN)-OXIDE EFFECT ON THE BRAIN GABA-ERGIC SYSTEM IN VITRO.] Ukr Biokhim Zh 1994;66(2):71-9. (Ukr)

Wide concentration range (10-14-10-4 M) of bis-(n-tributyltin)-oxide effect on Na+-dependent uptake, spontaneous and K+-stimulated release, specific receptor binding and GABA metabolism were studied in vitro experiments using brain slices, synaptic membrane fraction and brain tissue homogenates. It is shown that the dependence concentration-effect is of non-linear character in all cases. Prevailing supression of Na+-dependent uptake and specific receptor binding during K+-stimulated release and metabolism (production and utilization) of GABA activation were marked as a general tendency. Mechanisms of TBTO effect on the studied processes and the involvement of GABA-ergic system in realization of TBTO neurotoxic effects are discussed.

275
Kurek A, Ledwozyw A. [NEUROTOXIC ESTERASE ACTIVITY OF HEN'S BRAIN HOMOGENATES
IN VITRO.] Bromatol I Chemia Toksykol 1994;27(2):175-9. (Pol)

The usefulness of phenyl butyrate, phenyl valerate, and phenyl caproate as substrates for the determination of neurotoxic esterase activity, was ascertained. The activity of hen's brain homogenate neurotoxic esterase was measured, and I50 values of potent acetylcholinesterase inhibitors: mipafox and DFP (diisopropyl fluorophosphate), were determined.

276
Walker I, Coleman MD. THE BLOOD-BRAIN BARRIER: IN VITRO METHODS AND TOXICOLOGICAL APPLICATIONS. Toxicol In Vitro 1995;9(2):191-204.

The blood-brain barrier (BBB) is reviewed with reference to in vitro cell culture models and their use and potential use in toxicological studies. The structure, function and in vitro study of brain microvessel endothelial cells (BMEC) is briefly described, as well as the effects of a number of xenobiotics, such as solvents, metals, polycations and herbicides, on the viability and barrier function of the BBB model. The biotransformation of xenobiotics is increasingly thought to be responsible for many toxic reactions seen in living systems. Few studies have addressed the effects of the products of biotransformation on the integrity of the barrier model. Many of the specific human bioactivating enzymes, such as cytochrome P-450s, can now be conveniently studied in eukaryotic in vitro gene expression systems. The combination of such systems with a well characterized porcine BMEC culture

model might be useful in the study of reactive metabolites on the BBB, in terms of changes in indices of functional and structural BMEC viability. The potential applications and the value of such an experimental approach are discussed.

277
Samynathan YM, Bondy SC. INHIBITION OF PLASMA MEMBRANE AND MITOCHONDRIAL TRANSMEMBRANE POTENTIALS BY ETHANOL. Neurochem Res 1995;20(2):171-6.

The actions of ethanol and its primary oxidative metabolite, acetaldehyde, on plasma membrane and mitochondrial transmembrane potentials were examined in rat brain using fluorescence techniques. Subchronic treatment of adult rats with ethanol resulted in a significant depolarization of both the plasma and mitochondrial membranes when the mean blood ethanol level of the rats was 59 : 11 mM (mean : SEM, n = 6). Acute dosing of animals (4.5 g/kg, i.p.) failed to show any significant alterations. Various concentrations of ethanol, added in vitro to a crude synaptosomal preparation isolated from the rat cerebrocortex (P2) from untreated animals, depolarized both the plasma and mitochondrial transmembrane potentials in a dose-related manner. Addition of acetaldehyde in vitro did not reveal any significant effects on plasma or mitochondrial transmembrane potential.

278
Mokrzan EM, Kerper LE, Ballatori N, Clarkson TW. METHYLMERCURY-THIOL UPTAKE INTO CULTURED BRAIN CAPILLARY ENDOTHELIAL CELLS ON AMINO ACID SYSTEM L. J Pharmacol Exp Ther 1995;272(3):1277-84.

Recent in vivo studies suggest that the neurotoxin methylmercury (MeHg) is transported into brain as an L-cysteine complex by amino acid transport system L. To test this hypothesis, the mechanism of MeHg uptake into cultured calf brain capillary endothelial cells, an in vitro model of the blood-brain barrier, was examined. Uptake of Me203Hg-L-cysteine followed Michaelis-Menten kinetics, with a Km of 234 : 58 muM (mean : S.E.) and a Vmax of 57 : 25 pmolsec-1. Uptake of 10 muM MeHg-L-cysteine was stereoselective and Na+ independent and it was inhibited by the system L substrates L-leucine, 2-amino-2-norbornanecarboxylic acid and L-methionine (5 mM), consistent with transport of MeHg-L-cysteine by the L amino acid carrier. L-Glutamate and methylaminoisobutyric acid, which are transported by the acidic and A amino acid carriers, respectively, had no effect. Moreover, uptake of 3H-L-leucine (5 muM) was inhibited by 1 mM MeHg-L-cysteine. These observations provide direct evidence that MeHg-L-cysteine is transported into brain capillary endothelial cells by the L carrier. Uptake of other MeHg-thiols was also measured. MeHg-D, L-homocysteine uptake was 82 : 11% of MeHg-L-cysteine uptake, whereas uptakes of MeHg complexes of L-penicillamine, dimercaptosuccinic acid, N-acetyl-L-cysteine and glutathione were 57 t 16%, 19 : 7%, 10 : 4% and 8 : 5% of MeHg-L-cysteine uptake, respectively. These results illustrate the potential to minimize transport of MeHg across brain capillary endothelium by the careful choice of a thiol complexing agent.

279
Kowalski C, Crest M, Vuillet J, Pin T, Gola M, Nieoullon A. EMERGENCE OF A

SYNAPTIC NEURONAL NETWORK WITHIN PRIMARY STRIATAL CULTURES SEEDED IN SERUM-FREE MEDIUM. Neuroscience 1995;64(4):979-93.

In order to investigate the basic cellular mechanisms involved in neuronal interactions within the striatum, we prepared a primary striatal cell culture from rat fetal brain in chemically defined medium. Using morphological and whole-cell recording methods, we observed that an intensive neuritic elongation with a progressive build up of a sodium-dependent electrogenesis occurred during the first week of culture. Morphologically mature synapses began to develop after 10 days in vitro. By this time, most of the neurons (82 + 9%) received spontaneously synaptic potentials, which led them to fire (71 + 11%). The spontaneous firing was prevented by cadmium (200 muM) and tetrodotoxin (5 muM), which suggested that a Ca2+-dependent release of neurotransmitters was involved in the synaptic activation. We further obtained evidence that GABA, and to a lesser extent acetylcholine, contributed to these spontaneous synaptic potentials. At 15 days in vitro, it was possible to observe up to four synaptic contacts on a given dendrite. By this time, whole-cell recordings performed on pairs of neurons showed that the mature neurons were interconnected by excitatory synapses. As the number of synapses increased, the striatal neurons gradually formed a large network in which spontaneous activity developed, which tended to be organized into synchronized bursting patterns.

280
Green S. VALIDATION AND IN VITRO NEUROTOXICITY. Clin Exp Pharmacol Physiol 1995;22(5):383-4.

1. Validation of in vitro systems for studying neurotoxicants generally has not been accomplished, although in vitro tests have been used as screens to identify potential neurotoxic hazards and to study mechanisms. 2. A number of factors need to be taken into account when a test is validated: (i) a rationale for developing the test; (ii) clear biological or pathophysiological relevance of the endpoint to the effect detected in vivo; (iii) a standardized protocol and evidence of intra- and interlaboratory reproducibility; (iv) testing of chemicals representative of the categories of interest, including very toxic, moderately toxic and relatively non-toxic substances; and (v) a method to statistically evaluate the data. 3. Proper validation should lead to methods which can be used by regulatory agencies to make decisions regarding hazard/risk.

281
Capo MA, Alonso CE, Sevil MB, Frejo MT. "IN VITRO" EFFECTS OF METHYL-MERCURY ON THE NERVOUS SYSTEM: A NEUROTOXICOLOGIC STUDY.
J Environ Pathol Toxicol Oncol 1994;13(2):117-23.

Many of the currently prevailing toxicologic problems are due to the use of organic mercurial compounds in pesticides and fungicides. During recent years, environmental pollution has originated from the incorrect use of these organometals. Methyl-mercury (Me-Hg) is absorbed quickly from the gastrointestinal tract and is distributed to most tissues. The most important effect of Me-Hg is on the nervous tissue and is more relevant in the fetal

brain. We were interested in assessing the neurotoxic effects of Me-Hg on the central and peripheral nervous system. Neuronal cells cultures from 14-day-old fetal Wistar rats and ciliary ganglion cells cultures from 8-day-old chick embryos were used. Various Me-Hg concentrations (10-3 M to 10-8 M) were added to these cultures after 36 hr to study the morphologic changes. At 10-3 M and 10-4 M concentrations, cellular degeneration and death in the central nervous system (CNS) were noted. At 10-5 M concentrations, axonal and nerve fibers degeneration, loss of synapsis, and inhibition in the cellular development in CNS were seen; regroupment and destruction in the peripheral nervous system (PNS) was noted. Finally, at 10-6 M and 10-7 M concentrations, there were hardly any modifications in the CNS, whereas only the nervous processes were affected in the PNS.

282
Audesirk T, Shugarts D, Cabell-Kluch L, Wardle K. THE EFFECTS OF TRIETHYL LEAD ON THE DEVELOPMENT OF HIPPOCAMPAL NEURONS IN CULTURE. Cell Biol Toxicol 1995;11(1):1-10.

Triethyl lead is the major metabolite of tetraethyl lead, which is used in industrial processes and as an antiknock additive to gasoline. We tested the hypothesis that low levels of triethyl lead (0.1 nmol/L to 5 mumol/L) interfere with the normal development of cultured E18 rat hippocampal neurons, possibly through increases in intracellular free calcium ion concentration, (Ca2+)in. The study assessed survival and differentiation using morphometric analysis of individual neurons. We also looked at short-term (up to 3.75-h) changes in intracellular calcium using the calcium-sensitive dye fura-2. Survival of neurons was significantly reduced at 5 mumol/L, and overall production of neurites was reduced at : 2 mumol/L. The length of axons and the number of axons and dendrites were reduced at : 1 mumol/L. Neurite branching was inhibited at 10 nmol/L for dendrites and 100 nmol/L for axons. Increases in intracellular calcium were observed during a 3.75-h exposure of newly plated neurons to 5 mumol/L triethyl lead. These increases were prevented by BAPTA-AM; which clamps (Ca2+)in at about 100 nmol/L. Culturing neurons with BAPTA-AM and 5 mumol/L triethyl lead did not reverse the effects of triethyl lead, suggesting that elevation of (Ca2+)im is not responsible for decreases in survival and neurite production. Triethyl lead has been shown to disrupt cytoskeletal elements, particularly neurofilaments, at very low levels, suggesting a possible mechanism for its inhibition of neurite branching at nanomolar concentrations.

283
Walum E, Forsby A. MEASUREMENT OF CELL MEMBRANE TOXICITY BY MEANS OF 2-DEOXY-D-GLUCOSE. Methods Mol Biol 1995; 43:129-35.

284
Spoerri PE, Srivastava N, Vernadakis A. GABA ATTENUATES THE NEUROTOXIC EFFECTS OF ETHANOL IN NEURON-ENRICHED CULTURES FROM 8-DAY-OLD CHICK EMBRYO CEREBRAL
HEMISPHERES. Devel Brain Res 1995;86(1-2):94-100.

Neuron-enriched cultures were prepared from 8-day-old chick embryo cerebral

hemispheres and exposed to ethanol (50 mM), GABA (10-5 M) and ethanol (50 mM) + GABA (10-5 M) from day 4 to 8 in culture. At day 8, control, ethanol, GABA and ethanol + GABA-treated cultures were examined morphologically and biochemically. Choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) activities were used as markers for cholinergic and GABAergic neuronal phenotypic expression, respectively. Control cultures showed more numerous and large neuronal aggregates as well as prominent neuritic bundles. Moreover, cultures treated with GA-BA depicted even more numerous neuronal aggregates with interconnecting neurites as compared to control. In contrast, ethanol-treated cultures exhibited smaller neuronal aggregates with less prominent neuritic bundles than control. However, cultures treated concomitantly with ethanol + GABA exhibited numerous and larger aggregates than cultures treated with ethanol alone. Neuritic bundles which were highly reduced in ethanol-treated cultures became prominent in the presence of GABA. As previously reported, ethanol alone enhanced ChAT and reduced GAD activities. GABA given alone enhanced the expression of both neuronal phenotypes. When GABA was given concomitantly with ethanol the decline in GAD and the rise in ChAT observed in ethanol-treated cultures was restored by GABA to almost control levels. Thus, ethanol-induced alterations in morphology and neuronal phenotypes were counteracted by the neurontrophic effect of GABA.

285
Uto A, Dux E, Kusumoto M, Hossmann KA. DELAYED NEURONAL DEATH AFTER BRIEF HISTOTOXIC HYPOXIA IN VITRO. J Neurochem 1995;64(5):2185-92.

The effect of three metabolic inhibitors - iodoacetate, potassium cyanide, and potassium arsenate - on neuronal viability was studied in primary rat cortical and hippocampal CA1 neuronal cultures. iodoacetate (0.1 mM) applied for 5 min to 8-day-old cultures resulted in delayed neuronal death within 3-24 h in cortical and hippocampal CA1 neurons. Neuronal degeneration was preceded by transient inhibition of energy metabolism to synthesis tohe neuronal death were prevented by the free radical scavenger vitamin E but not by the glutamate antagonist MK-801. Removal of calcium during iodoacetate exposure could not protect against toxicity, and there was no increase of intracellular calcium concentration during and shortly after iodoacetate treatment. Cyanide and arsenate produced only partial neuronal degeneration, even at a dose of 10 mM. These observations demonstrate that brief exposure of neurons to low concentrations of iodoacetate produces a delayed type of neuronal death that is not mediated by either calcium or glutamate. The therapeutic effect of vitamin E points to a free-radical mediated injury and suggests that this type of pathology may also be involved in delayed neuronal death after transient energy depletion in vivo.

286
Wagner M, Toews AD, Morell P. TELLURITE SPECIFICALLY AFFECTS SQUALENE EPOXIDASE: INVESTIGATIONS EXAMINING THE MECHANISM OF TELLURIUM-INDUCED NEUROPATHY. J Neurochem 1995;64(5):2169-76.

A peripheral neuropathy characterized by a transient demyelinating/remyelinating sequence results when young rats are fed a

tellurium-containing diet. The neuropathy occurs secondary to a systemic block in cholesterol synthesis. Squalene accumulation suggested the lesion was at the level of squalene epoxidase, a microsomal monooxygenase that uses NADPH cytochrome P450 reductase to receive its necessary reducing equivalents from NADPH. We have now demonstrated directly specificity for squalene epoxidase; our in vitro studies show that squalene epoxidase is inhibited 50% in the presence of 5 muM tellurite, the presumptive in vivo active metabolite. Under these conditions, the activities of other monooxygenases, aniline hydroxylase and benzo(a)pyrene hydroxylase, were inhibited less than 5%. We also present data suggesting that tellurite inhibits squalene epoxidation by interacting with highly susceptible -SH groups present on this monooxygenase. In vivo studies of specificity were based on the compensatory response to feeding of tellurium. Following tellurium intoxication, there was up-regulation of squalene epoxidase activity both in liver (11-fold) and sciatic nerve (fivefold). This induction was a specific response, as demonstrated in liver by the lack of up-regulation following exposure to the nonspecific microsomal enzyme inducer, phenobarbital. As a control, we also measured the microsomal monooxygenase activities of aniline hydroxylase and benzo(a)pyrene hydroxylase. Although they were induced following phenobarbital exposure, activities of these monooxygenases were not affected following tellurium intoxication, providing further evidence of specificity of tellurium intoxication for squalene epoxidase.

287
Soderstrom S, Ebendal T. IN VITRO TOXICITY OF METHYL MERCURY: EFFECTS ON NERVE GROWTH FACTOR (NGF)-RESPONSIVE NEURONS AND ON NGF SYNTHESIS IN FIBROBLASTS. Toxicol Lett 1995;75(1-3):133-44.

The effect of methyl mercury chloride (MeHgCl) on the chick sympathetic and sensory dorsal root ganglia was studied in a biological in vitro assay. These cultures were not affected by the addition of MeHg up to a concentration of 2 muM. However, after an addition of 4-5 muM MeHg the capability of the neurons to respond to added nerve growth factor (NGF) was completely inhibited. The effect of MeHg was also examined in a fibroblast cell line, mouse 3T3 cells. After the addition of mercury to the culture medium at concentrations as low as 0.1 muM, an elevated production of the NGF protein was observed. However, NGF mRNA measured in the individual fibroblast cells by in situ hybridization was found to be reduced to about 80% of the control in the low level at day 2 of exposure. These results suggest that the release of NGF is actively enhanced from the 3T3 cells by addition of low levels of mercury. The results thus show that MeHg at low to moderate concentrations has adverse effects on NGF responses in cultured neurons and moreover alter levels of NGF production in cells, suggestive of mechanisms for mercury toxicity in the developing nervous system.

288
Stehrer-Schmid P, Wolf HU. EFFECTS OF BENZOFURAN AND SEVEN BENZOFURAN DERIVATIVES INCLUDING FOUR CARBAMATE INSECTICIDES IN THE IN VITRO PORCINE BRAIN TUBULIN ASSEMBLY ASSAY AND DESCRIPTION OF A NEW APPROACH FOR THE EVALUATION OF THE TEST DATA. Mutat Res 1995;339(1):61-72.

The influence of benzofuran and 7 benzofuran derivatives, including the carbamate insecticides benfuracarb, carbofuran, carbosulfan, and furathiocarb, on the in vitro assembly kinetics of porcine brain tubulin was investigated. A new approach to the evaluation of the raw data was made based on polynomial regression and the calculation of a polynomial function of the 11th degree fitting the raw data. By this procedure it is possible to calculate the parameters defining the shape of the absorbance curves and more parameters than those used so far can be included in the analysis of substance effects. In detail, the following curve parameters of the dependence of optical absorption on time were included in the evaluation of the substances of interest: the difference between maximum and minimum absorbance as a measure for the polymerization degree, the coordinates of the turning point of the curve, the slope of the tangent at the turning point which represents the maximum reaction velocity, the mean slope between the points with 10% absorbance increase and 90% absorbance increase and the duration of the lag phase. Out of the eight compounds tested, only the carbamate insecticides had distinct effects on the in vitro polymerization of tubulin, whereas benzofuran and the three 2,3-dihydro-2,2-dimethylbenzofuran derivatives without a carbamate function were inactive. Benfuracarb, carbofuran, carbosulfan, and furathiocarb led to a dose-dependent reduction of the polymerization degree of tubulin as well as to reduction of the maximum and mean reaction velocities. The strongest effects were obtained with furathiocarb and benfuracarb.

OCULAR TOXICITY

289

Earl LK, Jones PA, Dixit MB, O'Brien KA. COMPARISON OF FIVE POTENTIAL METHODS FOR ASSESSING OCULAR IRRITATION IN VITRO. Toxicol In Vitro 1995;9(3):245-50.

Thirty-three test substances comprising household and personal products and a pure surfactant were used in an independent evaluation of the ability of five selected in vitro assays to predict eye irritation potential. The data were compared with historical archived data from rabbit eye irritation tests conducted on the same subsamples. The data were assessed in three ways. The linear correlation for all 33 test substances with in vivo data was poor for each assay. However, the correlation improved greatly for some assays when a group of similar test substances was considered. Anal. of the data by Cooper's criteria suggested that fluorescein diacetate uptake by Chinese hamster V79 cells (V79) and the Microtox test kit were the best of the assays evaluated in discriminating correctly between the irritants and non-irritants that were identified by rabbit eye irritation testing. Some of the data were selected for further anal. because it was possible to predict differences, based on experience learned from animal data on similar substances, between the test substances without ref. to exptl. data. Near or more concd. test substances were predicted to more irritant than dilns. and an exptl. laundry powder formulation contg. 10% sodium metasilicate (MTS) was predicted to be more irritant than two analogous non-MTS contg. formulations. The exptl. data were then compared with the prediction. The in vivo rabbit eye irritation tests and Microtox distinguished correctly between all the test substances in this anal. Eytex was unable to distinguish correctly between any of the test substances in this anal. The other assays were able to distinguish between some of the

test substances. No single assay performed well in every type of anal. and it is concluded that a battery of assays is required to obtain reliable predictive data. Overall, Microtox, V79 fluorescein diacetate accumulation and 3T3 neutral red uptake were the most promising assays. Extreme caution should be used in comparing in vitro results with rabbit eye irritation data to make definitive conclusions.

290
Pasternak AS, Miller WM. FIRST-ORDER TOXICITY ASSAYS FOR EYE IRRITATION USING CELL LINES: PARAMETERS THAT AFFECT IN VITRO EVALUATION. Fundam Appl Toxicol 1995;25(2):253-63.

First-order toxicity assays can be used to rapidly screen test agents. Investigators in many labs. have used cultured cell lines to obtain correlations between first-order assay end-points and in vivo eye irritation (Draize test) for a wide variety of compds. Since validation is a key step in assay acceptance, it is important to understand which factors alter the responses of cell-line-based assays. In this study the authors examine: (1) the presence and configuration of a type I collagen gel; (2) the responses of epithelial (Sf-I-Ep) and fibroblast (Sirc and 3T3) cell lines; (3) the total glutathione content, ATP content, methionine incorporation, and neutral red absorption endpoint assays; (4) alc. (C2-C8), surfactant (Tween 20), and heavy metal (NiCl) test agents; and (5) test agent exposure time (1 to 24 h). The presence of a collagen gel and the cell type did not significantly affect endpoint assay R50 (test agent concn. that decreases assay response by 50%) values for a 1-h exposure to hexanol. The ATP and glutathione endpoints (after 1-h exposure) are able to distinguish between the relative in vivo toxicities of C2-C8 normal alcs. All four endpoint assays detected sublethal damage, with the ATP and methionine endpoints being the most sensitive. The type of test agent affects the endpoint response, as shown by the lack of a glutathione R50 value for a 1-h exposure to Tween 20 or NiCl. Even for a single test agent, endpoint assay R50 values may decrease continuously (ATP), decrease and then stabilize (glutathione), or remain unchanged (methionine incorporation) during a 24-h exposure.

291
Nishi C, Nakajima N, Ikada Y. IN VITRO EVALUATION OF CYTOTOXICITY OF DIEPOXY COMPOUNDS USED FOR BIOMATERIAL MODIFICATION. J Biomed Mater Res 1995;29(7):829-34.

The toxicity of various diepoxy compds. used for biomaterials crosslinking was investigated with a cell culture method and compared with an in vivo method. The neutral red uptake by cells was used to count the no. of cells still alive after contact with the diepoxy compds., because this method was more sensitive in cell counting than the other four methods studied in this work. The amt. of neutral red taken up by cells depended strongly on the activity of cells in comparison with other methods; only small amts. of neutral red were taken up when cells were in a low activity state even if they were still alive. The in vitro toxicity of diepoxy compds. evaluated by the neutral red method revealed a good correlation with that found by the in vivo Draize test. The in vitro cytotoxicity to a cell line of L929 was closely related to that

of primary culture cells of the normal rabbit cornea epidermal cell. The toxicity of diepoxy compds. was lower as their chain was longer, probably because of the lower chem. reactivity. All the diepoxy compds. investigated in this study exhibited lower cytotoxicity than formaldehyde, glutaraldehyde, and a water-sol. carbodiimide.

292
Rasmussen ES. PROSPECTS FOR USE OF IN VITRO METHODS FOR ASSESSMENT OF HUMAN
SAFETY. Levnedsmiddelstyr (National Food Agency of Denmark); 1995. Ministry of Health Publication No. 229. 126 p.

The prospects for use of in vitro methods as replacements for or supplements to animal expts. currently used for nonmedical assessment of human safety are discussed. The present report concs. on the potential introduction of scientifically validated in vitro alternatives to the acute toxicol. animal tests such as the ocular and dermal Draize tests for corrosivity and irritancy and the LD50 expts. for acute systemic effects.

293
Durrani AM, Farr SJ, Kellaway IW. INFLUENCE OF MOLECULAR WEIGHT AND FORMULATION pH ON THE PRECORNEAL CLEARANCE RATE OF HYALURONIC ACID IN THE
RABBIT EYE. Int J Pharm 1995;118(2):243-50.

Hyaluronic acid is a natural polymer which, due to its water retaining capability, binds to cell membranes and can therefore be considered as a putative vehicle for controlled ocular delivery. In an in vitro mucoadhesion test, the force of detachment was significantly greater for Healon (HA-Na) compared to low mol. wt. hyaluronic acid. Also, this bioadhesion was stronger for Healon at pH 5 than at pH 7.4. The precorneal clearance of sodium hyaluronate (0.2%) was investigated at pH 5.0 and 7.4 by employing gamma scintigraphic imaging of the 111In-labeled biopolymer. Protonation of the macromol. did not result in any increase in ocular mucoadhesion as the mean residence time at pH 5.0 was not significantly longer than at pH 7.4. The effect of mol. wt. of hyaluronic acid on the corneal retention was also investigated. There was a statistically significant difference ($p<0.05$) in the clearance half-life (t0.5) and AUC of % activity remaining vs time plot for Healon (mol. wt 2.2.times.106) compared to those obsd. for the two lower mol. wt. hyaluronic acid samples (mol. st 134 000 and 620 000).

294
Northover AM. THE USE OF THE BOVINE ISOLATED CORNEA AS A POSSIBLE IN VITRO TEST FOR OCULAR IRRITANCY. Methods Mol Biol 1995;43:205-10.

295
Spielmann H. HET-CAM TEST. Methods Mol Biol 1995;43:199-204.

296
Vian L, Vincent J, Maurin J, Fabre I, Giroux J, Cano JP. COMPARISON OF THREE IN VITRO CYTOTOXICITY ASSAYS FOR ESTINATING SURFACTANT OCULAR IRRITATION.

Toxicol In Vitro 1995;9(2):185-90.

Three in vitro cytotoxicity assays (neutral red uptake assay (NRU), MTT test and total protein content determination (TPC)) were analysed to assess their value for predicting the ocular irritancy potential of 20 surfactants. For each test, three established cell lines (SIRC rabbit corneal cells, Balb/c 3T3 and L929 mouse fibroblasts) were used. The concentration that induced 50% inhibition relative to controls (IC50) was calculated for each test, cell line and chemical. In vivo ocular irritancy data were compared with in vitro results. None of these assays provided a marked correlation of surfactant ocular irritation (Spearman or Pearson correlation coefficient lower than 0.65, P < 0.01 and moderate agreement with Kappa test). An IC50 threshold of 700 mug/ml was set to discriminate between surfactant irritation and non-irritation. Three compounds were detected as false negatives (CHAPS, CHAPSO and sodium taurocholate), and two as false positives (Triton X155 and Brij 35).

297
Joller PW, Coquette A, Noben J, Pirovano R, Southee JA, Logemann PK. EUROPEAN INTERLABORATORY EVALUATION OF AN IN VITRO OCULAR IRRITATION MODEL SKIN-2
MODEL ZK1100 USING 18 CHEMICALS AND FORMULATED PRODUCTS. In: Reinhardt CA, editor. Alternatives to Animal Testing: New Ways in the Biomedical Sciences, Trends and Progress; Symposium; 1992 Nov 30; Zurich, Switzerland. New York: VCH Publishers, Inc: 1994. p. 159-64.

PULMONARY TOXICITY
298
Herman EH, Hasinoff BB, Zhang J, Raley LG, Zhang TM, Fukuda Y, Ferrans VJ. MORPHOLOGIC AND MORPHOMETRIC EVALUATION OF THE EFFECT OF ICRF-187 ON BLEOMYCIN-INDUCED PULMONARY TOXICITY. Toxicology 1995;98(1-3):163-75.

Morphologic and morphometric studies were made of the protective effects of ICRF-187 against the pulmonary damage induced by bleomycin in male and female C57/BL6 mice. Sixty minutes prior to the subcutaneous administration of 15 mg/kg of bleomycin, animals received either saline or ICRF-187 (300 or 150 mg/kg) intraperitoneally, twice a week for 4 weeks. The lungs of animals treated with bleomycin alone showed inflammation, hyperplasia of type II epithelial cells, squamous cell metaplasia and fibrosis. The extent of fibrosis was quantified by means of a color videometric system and histologic sections of lung stained according to a modified Masson trichrome method. The severity of these alterations, particularly of fibrosis, was reduced in all groups of animals pretreated with ICRF-187. The fibrosis was reduced to a similar extent in female mice treated with the 300 mg/kg and the 150 mg/kg doses of ICRF-187, from 39.3% to 17.6% and 13.3%, respectively. ICRF-187 induced significantly different degrees of reduction in fibrosis in the 2 groups of male mice treated with the 150 mg/kg and the 300 mg/kg doses, from 30% to 19.7% and 12.2%, respectively. In vitro studies indicated that both ICRF-187 and its open-ring hydrolysis product (ADR-925) remove iron slowly from the bleomycin-iron complex. This observation provides a basis for the

concept that ICRF-187 protects by chelating iron involved in the formation of the bleomycin-Fe3+ complex that generates reactive oxygen radicals capable of causing pulmonary damage.

RENAL AND HEPATIC TOXICITY

299

Lave T, Schmitt-Hoffmann AH, Coassolo P, Valles B, Ubeaud G, Ba B, Brandt R, Chou RC. A NEW EXTRAPOLATION METHOD FROM ANIMALS TO MAN: APPLICATION TO A METABOLIZED COMPOUND, MOFAROTENE. Life Sci 1995; 56(26):PL473-PL478.

Allometric scaling (a technique which uses data obtained in lab. animals to predict human pharmacokinetics) works well for drugs that are cleared intact, but is less successful with extensively metabolized compds. This paper describes a new method to improve the accuracy of such projections, by integrating metabolic data obtained in vitro (e.g., with liver microsomes or hepatocytes) into these calcns. The approach was used prospectively, to predict the clearance of mofarotene (Ro 40-8757) in humans from in vivo kinetic data obtained in mice, rats and dogs. This compd. was selected to illustrate this approach because it is exclusively eliminated through metab. Without the metabolic correction and by using brain wt. as an empirical correcting factor, the oral clearance values predicted for man were 2.7 and 0.6 mL/min/kg, resp. This fell outside the range subsequently obtained in healthy volunteers treated orally with 300 mg mofarotene (7.5 .+-. 4.0 mL/min/kg). However, inclusion of the microsomal or hepatocyte data gave values of 5.1 and 4.2 mL/min/kg, resp., illustrating that the integration of in vitro metabolic data improves the accuracy of kinetic extrapolations. In contrast to the existing empirical techniques, this approach offers a rational basis for predicting clearance of metabolized compds. in humans.

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Khan SA, Ghosh S, Wickstrom M, Miller LA, Hess R, Haschek WM, Beasley VR. COMPARATIVE PATHOLOGY OF MICROCYSTIN-LR IN CULTURED HEPATOCYTES, FIBROBLASTS,
AND RENAL EPITHELIAL CELLS. Nat Toxins 1995;3(3):119-28.

The cyanobacterial toxin microcystin-LR (MCLR) is a potent inhibitor of protein phosphatases 1 and 2A, and is selectively toxic to the liver in vivo and to isolated hepatocytes in vitro. This selectivity is believed to be due to toxin uptake via bile acid carriers. We investigated at the light and ultrastructural levels the effects of high concentrations of MCLR and long incubation times to determine in vitro whether fibroblasts and kidney cells (non-target cells) respond in the same manner as do hepatocytes (target cells) at low concentrations and short incubation times. Cultured rat skin fibroblasts (ATCC 1213) and rat kidney epithelial cells (ATCC 1571) were incubated with MCLR at 133 muM for 1-24 hr. Lesions in these cells were compared with those in cultured hepatocytes incubated with MCLR at 13.3 muM from 1 to 32 min. Lesions in hepatocytes, kidney cells, and fibroblasts were noted at 4 min, 1 hr, and 8 hr, respectively, after initial exposure to MCLR. Lesions in all three cell types progressed and included plasma membrane blebbing, loss of cell-to-cell contact, clumping and rounding of cells,

cytoplasmic vacuolization, and redistribution of cytoplasmic organelles. Loss of microvilli, whorling of rough endoplasmic reticulum, dense staining and

dilated cristae in mitochondria, and pinching off of membrane blebs were noted only in hepatocytes. Nuclear changes typical of apoptosis were observed only in fibroblasts and kidney cells. Similarities in responses of different cell types to MCLR exposure probably reflect a common biochemical mechanism of action, i.e., inhibition of protein phosphatases 1 and 2A as described by others. The observed differences in the responses of the cell types examined in this study may reflect differences in the proteins phosphorylated and the severity of hyperphosphorylation.

301
Ferro M. HEPATOMA CELL CULTURES AS IN VITRO MODELS FOR HEPATOTOXICITY. Methods Mol Biol 1995;43:51-7.

302
James NH, Roberts RA. SPECIES DIFFERENCES IN THE CLONAL EXPANSION OF HEPATOCYTES IN RESPONSE TO THE COACTION OF EPIDERMAL GROWTH FACTOR AND NAFENOPIN, A RODENT HEPATOCARCINOGENIC PEROXISOME PROLIFERAR. Fundam Appl
Toxicol 1995;26(1):143-9.

Peroxisome proliferators are members of the nongenotoxic family of rodent hepatocarcinogens. There exist substantial species differences in response to peroxisome proliferators among mammalian species. We have reported previously that peroxisome proliferators can synergize with epidermal growth factor (EGF) to promote the clonal expansion of rat hepatocytes associated with the early stages of hepatocarcinogenesis. The aim of the present study was to determine whether responsiveness in this in vitro assay reflected the known species differences in response to peroxisome proliferators. The process of tumorigenicity was modeled in the soft agar cloning assay since growth in soft agar is thought to reflect the early stages of tumorigenesis. This is because clonal expansion under these conditions requires the cells to survive, to undergo mitosis, and to escape from the contact-dependent growth associated with normal cell behavior. The data presented here show that mouse hepatocytes are able to undergo clonal expansion in soft agar in response to nafenopin and EGF giving a three- to fourfold increase in colony numbers over control. This result is comparable to the fivefold increase in rat hepatocyte colony numbers that we have reported previously. In contrast, hamster, guinea pig, and human hepatocytes did not respond to the concerted action of EGF and nafenopin despite their ability to respond to EGF as a mitogen in monolayer culture. These data demonstrate that the clonal expansion of rodent hepatocytes in soft agar in response to peroxisome proliferators and EGF displays the same species differences as other pleiotropic responses to these compounds and is likely therefore to be relevant to the process of hepatocarcinogenesis.

303
Shen HM, Ong CN, Shi CY. INVOLVEMEMT OF REACTIVE OXYGEN SPECIES IN AFLATOXIN

B1-INDUCED CELL INJURY IN CULTURED RAT HEPATOCYTES. Toxicology

1995;99(1-2):115-23.

The role of reactive oxygen species (ROS) in AFB1-induced cell injury was investigated using cultured rat hepatocytes. Malonaldehyde (MDA) generation and lactate dehydrogenase (LDH) release were determined as indices of lipid peroxidation and cell injury, respectively. Exposure to AFB1 for up to 72 h resulted in significantly elevated levels of LDH being released into the medium as well as the MDA generation in cultured hepatocytes. These effects were dose-dependent, indicating that AFB1 was capable of inducing oxidative damages in the cell. Further, MDA generation and LDH release were effectively inhibited by the addition of the following: (1) superoxide dismutase (500 units/ml), (2) catalase (1500 units/ml), (3) 10 mM desferrioxamine (a specific iron chelator), or (4) 260 mM dimethyl sulfoxide (a hydroxyl radical scavenger). These evidences therefore suggest that ROS, such as superoxide radicals, hydroxyl radicals and hydrogen peroxides, are involved in AFB1-induced cell injury in cultured rat hepatocytes.

304
Steinmassl D, Pfaller W, Gstraunthaler G, Hoffmann W. LLC-PK1 EPITHELIA AS A MODEL FOR IN VITRO ASSESSMENT OF PROXIMAL TUBULAR NEPHROTOXICITY. In Vitro Cell Dev Biol Anim 1995;31(2):94-106.

LLC-PK1 cells, an established epithelial cell line derived from pig kidney, were used as a model system for assessment of nephrotoxic side effects of three cephalosporin antibiotics: cephaloridine, ceftazidime, and cefotaxime. Toxic effects of these xenobiotics were monitored on confluent monolayers by light and electron microscopy and by the release of cellular marker enzyme activities into the culture medium. In addition, LLC-PK1 cells were grown on microporous supports, and cephalosporin-induced alteration of epithelial functional integrity was monitored by a novel electrophysiologic approach. For this purpose, an Ussing chamberlike experimental setup was used. The dose-dependent effects on transepithelial ionic permselectivity were monitored under conditions in which defined fractions of the apical culture medium NaCl contents were replaced iso-osmotically by mannitol. This method of determining the functional intactness of the epithelial barrier by measuring dilution potentials was found to be far more sensitive than monitoring cell injury by means of morphology or measurement of enzyme release. As expected from animal experimental data, a dose-dependent disruption of monolayer integrity was detected with all three methodologies applied. Cephaloridine was found the most toxic compound followed by ceftazidime, where a 3-fold, and cefotaxime, where a 10-fold dose of that of cephaloridine was needed to produce cell injury. Measurement of transepithelial dilution potentials was more sensitive as compared to the release of the apical plasma membrane marker enzyme activities alkaline phosphatase and gamma-glutamyltranspeptidase, the cytosolic lactate dehydrogenase, or the mitochondrial glutamate dehydrogenase. The data were compared to the effects of the aminoglycoside antibiotic gentamicin, which at least with respect to its effects on LLC-PK1 morphology and enzyme release, but not transepithelial electrical properties, was already investigated.

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Nath KA, Balla J, Croatt AJ, Vercellotti GM. HEME PROTEIN-MEDIATED RENAL INJURY: A PROTECTIVE ROLE FOR 21-AMINOSTEROIDS IN VITRO AND IN VIVO. Kidney Int 1995;47(2):592-602.

21-aminosteroids ("lazaroids") have recently excited much interest by virtue of their ability to inhibit lipid peroxidation in vitro and to protect against neural injury in vivo. We tested the effect of these compounds in models of heme protein-mediated renal injury in vitro and in vivo. We devised an in vitro model of heme protein-induced toxicity in which renal epithelial cells were exposed to heme proteins for one hour, after which they were subjected to glutathione depletion by 1-chloro-2,4-dinitrobenzene (CDNB). This model was associated with more than a threefold increase in lipid peroxidation (as measured by thiobarbituric acid reactive substances, TBARS) and a marked reduction in cellular glutathione content. In this model, 21-aminosteroids virtually prevented cytotoxicity as measured by the 51-chromium release assay, and significantly reduced TBARS in a dose-dependent manner. Catalase was partially protective in this model, thereby indicating hydrogen peroxide-dependent toxicity. While pursuing mechanisms accounting for enhanced cellular generation of hydrogen peroxide, we uncovered the first direct evidence that the heme prosthetic group per se directly stimulates cellular generation of hydrogen peroxide; complementing these findings is the remarkable efficacy of 21-aminosteroids in protecting against cytotoxicity induced by hydrogen peroxide. We also tested the capacity of 21-aminosteroids to protect against heme protein-mediated renal injury in vivo. Prior administration of 21-aminosteroids attenuated reductions in GFR and renal blood flow rates following the systemic infusion of methemoglobin in normal rats. 21-aminosteroids also attenuated renal injury observed over three successive days in the glycerol model of heme protein-mediated injury when this model was induced at a higher dose of glycerol (8 ml/kg body wt) but not at a lower dose (5 ml/kg body wt). We conclude that 21-aminosteroids protect against heme protein-mediated renal injury in vitro and in vivo. We suggest that these compounds are potentially useful in such clinical conditions as rhabdomyolysis, intravascular hemolysis and renal injury associated with hemoglobin-based red blood cell substitutes.

306
Vickers AE. USE OF HUMAN ORGAN SLICES TO EVALUATE THE BIOTRANSFORMATION AND
DRUG-INDUCED SIDE-EFFECTS OF PHARMACEUTICALS. Cell Biol Toxicol 1994;10(5-6):407-14.

Human liver and kidney organ slices were used to investigate the biotransformation competence of the slices in combination with several markers of cell viability and function. The immunosuppressant cyclosporin A (CSA) is extensively metabolized in liver slices to the three known primary metabolites and many secondary metabolites. In kidney cortex slices the biotransformation of CSA is far more pronounced in humans than in rats. In human liver slices, levels of CYP3A, the proteins metabolizing CSA, are depressed about 25% by 1 and 10 mumol/L CSA within 24 h, indicating that high blood or tissue concentrations will inhibit CSA clearance. A clinical marker for liver damage is the release of cellular alpha-glutathione-S-transferases (alpha GST). In

this study the alpha GST levels were used to assess donor organ quality, organ slice incubation conditions, and compound exposure. A marker for cell death in human cells is the solubilization and release of nuclear matrix proteins (Numa). Increases were apparent only after 48 h of culture. A side-effect of CSA is that it induces hypertension and perturbs the lipid profile of transplant recipients. A potential marker for lipid disturbances is levels of serum lipoprotein (a) (Lp(a)), which is synthesized in the liver and found only in humans, apes, and nonhuman primates. CSA increases Lp(a) levels in the human liver slice cultures about 2-fold. This study has demonstrated that the biotransformation capability of the organ slices contributes to the optimization of the in vitro system and to the evaluation of markers for drug induced side-effects or toxicity.(ABSTRACT TRUNCATED AT 250 WORDS)

307
White DJ, Seaman C. LLC-RK1 CELL SCREENING TEST FOR NEPHROTOXICITY. Methods Mol Biol 1995;43:11-6.

308
Knapek DF, Serrano M, Beach D, Trono D, Walker CL. ASSOCIATION OF RAT P15INK4B/p16INK4 DELETIONS WITH MONOSOMY 5 IN KIDNEY EPITHELIAL CELL LINES BUT
NOT PRIMARY RENAL TUMORS. Cancer Res1995;55(8):
1607-12.

Recently the putative tumor suppressor gene p16INK4 was mapped to human chromosome 9p21, which is homologous to rat chromosome 5. Monosomy of rat chromosome 5 occurs with high frequency in rat kidney tumor-derived cell lines (ERC lines). Thus, we studied these lines in order to investigate the involvement of p15INK4B in the genesis of this tumor type. p15INK4B and p16INK4 were found by Southern blot analysis to be codeleted in five of seven of these lines. This was confirmed by Northern blot analysis with a probe for the rat p15INK4B gene. In normal rat tissues, expression of p15INK4B was abundant in lung (2.5 and 2.0 kilobases), less abundant in testis (2.5, 2.0, 1.1, and 0.9 kilobases), barely detectable in liver (2.0 kilobases), and not detectable in neonatal kidney, adult kidney, brain, heart, or spleen. In the ERC lines, p15INK4B was expressed as a single 2.0-kilobase transcript observed only in those cell lines in which the gene was detected by Southern blot analysis. However, neither p15INK4B nor p16INK4 were deleted in 12 of 12 primary kidney tumors examined, suggesting that deletion of these genes is not directly involved in the process of renal tumor development but may be related to tumor progression or autonomous growth in vitro. A panel of rat kidney epithelial cell lines chemically transformed in vitro (TRKE lines) that had high-frequency monosomy 5 were also examined, but deletion of p15INK4B and p16INK4 was observed in only one of six of the TRKE lines. To our knowledge, this is the first reported investigation of these genes in rodent tumors and cell lines, and its data support the theory that alterations of genes located in the INF region of rat chromosome 5 may play a role in rodent cell transformation.

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Rankin GO, Valentovic MA, Nicoll DW, Ball JG, Anestis DK, Brown PI, Hubbard

JL. ACUTE RENAL AND HEPATIC EFFECTS INDUCED BY 3-HALOANILINES IN THE FISCHER
344 RAT. J Appl Toxicol 1995;15(2):139-46.

Haloanilines are commonly used as chemical intermediates in the manufacture of a wide range of products. The purpose of this study was to examine the in vivo nephrotoxic and hepatotoxic potentials of the 3-haloanilines. The in vitro effects of the 3-haloanilines on renal function were also examined. In the in vivo experiments, male Fischer 344 rats (four rats/group) were administered a single intraperitoneal (i.p.) injection of an aniline hydrochloride (1.0 or 1.25 mmol kg-1) or vehicle. Renal and hepatic function were monitored at 24 and/or 48 h post-treatment. None of the 3-haloanilines were potent nephrotoxicants at either dose level. The greatest effects on renal function were observed following administration of 3-chloroaniline at a dose of 1.25 mmol kg-1 (oliguria, glucosuria, hematuria, decreased p-aminohippurate accumulation by renal cortical slices and increased blood urea nitrogen concentration). 3-Chloroaniline also was the only aniline compound to increase plasma ALT/GPT activity at 48 h. In the in vitro experiments, the ability of ananiline (10-5-10-3 M) to decrease organic ion accumulation in renal cortical slices from untreated rats was examined. The decreasing order of in vitro nephrotoxic potential was 3-iodoaniline > 3-bromoaniline > 3-chloroaniline > aniline > 3-fluoroaniline. These results indicate that the 3-haloanilines are not potent nephrotoxicants or hepatotoxicants at sublethal doses. In addition, the reasons why the 3-haloanilines have different orders of nephrotoxic potential in vivo and in vitro are not clear at this time.

310
Valentovic MA, Ball JG, Anestis DK, Rankin GO. COMPARISON OF THE IN VITRO TOXICITY OF DICHLOROANILINE STRUCTURAL ISOMERS. Toxicol In Vitro 1995;9(1):75-81.

Acute exposure to certain dichloroaniline (DCA) isomers results in renal and hepatic toxicity in vivo. In the present study we examined whether dichloroaniline structural isomers were cytotoxic to liver and kidney slices in vitro and compared the toxicities of the different structural isomers. These studies were necessary in order to validate the use of an in vitro slice system for examination of the cellular mechanisms for toxicity. Renal cortical and hepatic slices were incubated for 90 min with 2,3-DCA, 2,4-DCA, 2,5-DCA, 2,6-DCA, 3,4-DCA or 3,5-DCA at a final concentration of 0-1 mM. Pyruvate-directed gluconeogenesis was measured following an additional 30-min incubation with 10 mM pyruvate. Cytotoxicity was also determined by measurement of lactate dehydrogenase (LDH) release 120 min after the addition of dichloroaniline isomers at a final concentration of 0, 0.5, 1 or 2 mm. Gluconeogenesis in renal cortical slices was inhibited by all of the isomers beginning at a concentration of 0.5 mM. Renal slice LDH leakage was elevated above control levels by 1-2 mm 3,4-DCA or 3,5-DCA. A final concentration of 2 mM was needed for 2,3-DCA, 2,4-DCA, 2,5-DCA or 2,6-DCA in order to detect a significant ($P < 0.05$) increase in renal slice LDH leakage. Hepatic slices

incubated with 0.5-2 mM 2,3-DCA or 2 mm 2,5-DCA exhibited diminished pyruvate-directed gluconeogenesis. After exposure to 2,4-DCA, 2,6-DCA, 3,4-DCA

or 3,5-DCA, pyruvate-directed gluconeogenesis was similar to that in the controls. LDH leakage was increased significantly ($P < 0.05$) above control values by exposure to 2 mm 3,4-DCA or 3,5-DCA. In conclusion, DCA structural isomers were toxic in vitro to liver and kidney slices. These results indicated that the kidney was more sensitive than the liver to DCA isomers, and that the most toxic isomer was 3,5-DCA. These results are similar to those previously observed in vivo.

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Willinger CC, Thamaree S, Schramek H, Gstraunthaler G, Pfaller W. IN VITRO NEPHROTOXICITY OF RUSSELL'S VIPER VENOM. Kidney Int 1995;47(2):518-28.

To assess direct nephrotoxicity of Russell's viper venom (RVV; Daboia russelii siamensis), isolated rat kidneys were perfused in single pass for 120 min. Ten mug/ml and 100 mug/ml RVV were administered 60 minutes and 80 minutes, respectively, after starting the perfusion. Furthermore, cultured mesangial cells and renal epithelial LLC-PK1 and MDCK cells were exposed to RVV (100 to 1000 mug/ml) for 5 minutes up to 48 hours. The IPRK dosedependently exhibited reductions of renal perfusate flow (RPF, 7.7 : 2.4 vs. 16.5 : 0.7 ml/min g kidney wt in controls, experimental values given are those determined 10 minutes after termination of 100 mug/ml RVV admixture), glomerular filtration rate (GFR 141 : 23 vs. 626 : 72 mul/min g kidney wt) and absolute reabsorption of sodium (TNa 8 : 1.7 vs. 79 : 9 mumol/min g kidney wt), and an increased fractional excretion of sodium (FENa 60 : 7 vs. 8 : 0.8%) and water (FEH2O 68 : 3.2 vs. 13 : 1.2%). Urinary flow rate (UFR) showed both oliguric and polyuric phases. Functional alterations of this type are consistent with ARF. Light and electron microscopy of perfusion fixed IPRK revealed an extensive destruction of the glomerular filter and lysis of vascular walls. Various degrees of epithelial injury occurred in all tubular segments. In cell culture studies RVV induced a complete disintegration of confluent mesangial cell layers, beginning at concentrations of 200 mug/ml. In epithelial LLCPK, and MDCK cell cultures only extremely high doses of RVV (>600 and 800 mug/ml, respectively) led to microscopically discernible damage. These results clearly demonstrate a direct dose dependent toxic effect of RVV on the IPRK, directed primarily against glomerular and vascular structures, and on cultured mesangial cells.

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Toyokuni S, Sagripanti JL, Hitchins VM. CYTOTOXIC AND MUTAGENIC EFFECTS OF FERRIC NITROLOTRIACETATE ON L5178Y MOUSE LYMPHOMA CELLS. Cancer Lett 1995;88(2):157-62.

An iron chelate, ferric nitrilotriacetate (Fe-NTA), induces renal proximal tubular necrosis that leads to a high incidence of renal adenocarcinoma in rodents. Others have shown that Fe-NTA induces modified DNA base products both in vitro and in vivo. However, Fe-NTA is negative in the Ames Salmonella test with or without S9 activation. The goal of this project was to determine if Fe-NTA is cytotoxic and mutagenic using the L5178Y (TK +/-) mouse lymphoma assay. Our experiments showed a relationship between the concentration of Fe-NTA (0 to 1 mM) and the decrease in relative survival. An exposure-dependent increase in the number of mutations was observed with

increasing concentrations of Fe-NTA. At 14% relative survival, there was about a 4-fold increase in mutations (trifluorothymidine resistance) over unexposed, control cells. Ferric nitrate or nitrilotriacetic acid alone induced a relatively low 1.5- or 1.1-fold increase in mutation, respectively. Our results establish that Fe-NTA is mutagenic in the L5178Y mouse lymphoma assay system.

313
James PE, Jackson SK, Grinberg OY, Swartz HM. THE EFFECTS OF ENDOTOXIN ON OXYGEN CONSUMPTION OF VARIOUS CELL TYPES IN VITRO: AN EPR OXIMETRY STUDY. Free Radic Biol Med 1995;18(4):641-7.

We have studied the effects of bacterial endotoxin on the oxygen consumption of a variety of target cells, and found that the rate of utilization of oxygen by treated cells was decreased in a time- and dose-dependent manner. Precise EPR measurement of oxygen concentrations enabled us to demonstrate that this effect was linked to mitochondrial dysfunction and was particular to each cell type. Such detailed knowledge on oxygen utilization by viable whole cells and the varied effects of endotoxin are as yet undocumented. Oxygen consumption was shown to decrease quite markedly in CHO cells and kidney cells from the cortex region. Cells from the kidney medulla region had lower baseline consumption and were stimulated to increased levels of oxygen consumption on addition of similar doses of endotoxin. Macrophages exhibited a dual response in that in addition to inhibiting mitochondrial oxygen consumption, endotoxin pretreatment primed these cells to exhibit an enhanced oxidative burst on stimulation with Zymosan. These results show that endotoxin has a direct effect on normal cellular oxygen consumption and is an important parameter that must be considered when following the early effects on cells and tissues during the septic syndrome.

314
Mattana J, Singhal PC. HEPARIN ATTENUATES THE EFFECT OF MITOGENIC VASOCONSTRICTORS ON MESANGIAL CELL PROLIFERATION AND HANDLING OF IMMUNOGLOBULIN G COMPLEXES. J Pharmacol Exp Ther 1995;273(1):80-7.

Angiotensin II (ANG II) and endothelin-1 (ET-1) may both play significant roles in causing mesangial expansion and glomerulosclerosis after renal injury by enhancing mesangial cell (MC) proliferation and by increasing MC uptake of macromols. Heparin has been demonstrated to significantly ameliorate the development of glomerulosclerosis in animal models of progressive renal injury, although the mechanism is unknown. We undertook the present study of examine in an in vitro system the mechanism by which heparin might modulate the effects of ANG II and ET-1 on MC proliferation and MC uptake of IgG complexes. Heparin was found to suppress MC uptake of IgG complexes in a concn.-related manner. Whereas ANG II significantly enhanced uptake of IgG complexes, this increase was significantly antagonized by coincubation of MC with heparin at therapeutic concn. (1 U/mL). ET-1 also was found to significantly increase MC uptake of IgG complexes, and this increase also was significantly antagonized by coincubation with heparin. Heparin was found to inhibit surface binding of IgG to MC in a concn.-related manner (55% redn. at 1 U/mL). MC [3H]thymidine incorporation was significantly enhanced by both ANG

II and ET-1 and these mitogenic effects were significantly attenuated by coincubation of cells with heparin (and, resp.). The antiproliferative effect of heparin was attenuated by the nitric oxide synthase inhibitor Nomega-nitro-L-arginine methylester and by the guanylate cyclase inhibitor methylene blue suggesting that a nitric oxide-cGMP-dependent mechanism may account for the antiproliferative effect of heparin. By using the diazotization assay, heparin significantly increased MC nitric oxide prodn. in a concn.-related manner. These data demonstrate that heparin can antagonize the mitogenic effects of ANG II and ET-1 on MC as well as the ability of these agents to enhance MC uptake of IgG complexes. These effects may play a role in the obsd. ability of heparin to afford protection against progressive glomerulosclerosis of renal injury.

REPRODUCTIVE TOXICITY

315
Davies WJ, Freeman SJ. THE HYDRA ATTENUATA ASSAY. Methods Mol Biol 1995;43:321-6.

316
Davies WJ, Freeman SJ. THE DROSOPHILA MELANOGASTER ASSAY. Methods Mol Biol 1995;43:317-20.

317
Davies WJ, Freeman SJ. FROG EMBRYO TERATOGENESIS ASSAY. Methods Mol Biol 1995;43:311-6.

318
Davies WJ, Freeman SJ. CHICK EMBRYOTOXICITY SCREENING TEST CHEST I AND II. Methods Mol Biol 1995;43:307-10.

319
Cosenza ME, Bidanset J. EFFECTS OF CHLORPYRIFOS ON NEURONAL DEVELOPMENT IN RAT
EMBRYO MIDBRAIN MICROMASS CULTURES. Vet Hum Toxicol 1995;37(2):118-21.

320
Brunstrom B, Engwall M, Hjelm K, Lindquist L, Zebuehr Y. EROD INDUCTION IN CULTURED CHICK EMBRYO LIVER: A SENSITIVE BIOASSAY FOR DIOXIN-LIKE ENVIRONMENTAL POLLUTANTS. Environ Toxcol Chem 1995;14(5):837-42.

A technique for studying 7-ethoxyresorufin O-deethylase (EROD) induction in chick embryo liver in vitro was developed. Livers from 8-d-old embryos were cultured in rotating vials at 37~ C for 48 h in a medium to which DMSO-dissolved test compounds had been added. This bioassay proved to be highly sensitive to dioxin-like compounds, and its usefulness for assessing the toxic potency of such compounds in environmental samples was demonstrated. Concentration-response curves were determined for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3,3'4,4',5-pentachlorobiphenyl (PCB IUPAC no. 126), 3,3',4,4'-tetrachlorobiphenyl (PCB 77), 2,3,3',4,4'-pentachlorobiphenyl (PCB 105), and benzo(k)fluoranthene (BkF).

TCDD induced EROD in a concentration-dependent manner, having an EC50 of 5.0-12 M. The cultured embryo livers were extremely sensitive to TCDD, and about 30 fg of this compound per liver (2as enough to significantly induce EROD. The EC50 values obtained for PCBs 126, 77, 105, and BkF were 4.4 M, 1.6nduction levels obtained for three different preparations of polychlorinated naphthalenes (PCNs) were less than those of the other compounds tested. When the technical PCN mixture Halowax 1014 was coadministered with TCDD, the induction was lower than that caused by TCDD alone. An organic extract of fly ash from a municipal waste combustion plant was very potent. Considering its contents of polychlorinated dibenzo-p-dioxins/furans, expressed as TCDD equivalents, the EC50 obtained was close to that for TCDD.

321
Mothersill C. HUMAN ESOPHAGEAL CULTURE. Methods Mol Biol 1995;43:75-9.

322
Mclean M, Watt MP, Berjak P, Dutton MF. AFLATOXIN B1: ITS EFFECTS ON AN IN VITRO PLANT SYSTEM. Food Addit Contam 1995;12(3):435-43.

The phytotoxic effects of aflatoxin B1 (AFB1) on in vitro cultures of differentiating calli and regenerating plantlets of Nicotiana tabacum were assessed. Callus appeared more sensitive to the effects of AFB1, with fresh mass accumulation and callus chlorophyll levels affected at low (approximately 0.5 mug/ml) aflatoxin concentrations. Transmission electron microscopy revealed early deteriorative alterations in chloroplast morphology. Inhibitory effects of the toxin (up to and including 10 mug/ml) on callus fresh mass accumulation were reversed following a 3 week toxin-free recovery period. In tobacco plantlets, root and leaf development, and root and leaf mass were significantly inhibited in a dose-dependent fashion with increasing AFB1 concentration above 0.5 mug/ml. Inhibitory effects on plantlet root development were more pronounced that on leaf development.

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Peters JM, Duncan JR, Wiley LM, Keen CL. INFLUENCE OF ANTIOXIDANTS ON CADMIUM TOXICITY OF MOUSE PREIMPLANTATION EMBRYOS IN VITRO. Toxicology 1995;99(1-2):11-8.

To test the hypothesis that the developmental toxicity of cadmium (Cd) is due in part to oxidative damage, embryos were cultured in medium containing 0.0, 1.0, 3.0, or 6.0 muM Cd with or without various antioxidants for 72 h. Ascorbate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and glutathione (GSH) were all effective at ameliorating 1.0 muM Cd-induced embryotoxicity. For embryos cultured in medium containing either 3.0 or 6.0 muM Cd, GSH was effective at ameliorating Cd toxicity while the other antioxidants tested were ineffective. Pretreating embryos with antioxidants for 24 h prior to exposing them to Cd and antioxidants did not significantly alter the previously observed improvement with the exception that pretreatment with GSH virtually eliminated Cd-induced embryotoxicity between 1.0 and 6.0 muM Cd. A 4-h exposure to GSH prior to culture in Cd markedly improved embryo development suggesting that GSH taken up during pretreatment can provide

protection against Cd-induced embryotoxicity. This work supports the hypothesis that the developmental toxicity of Cd is in part due to oxidative damage that can be modulated by select antioxidants.

324
Kishimoto T, Oguri T, Tada M. METHYLMERCURY-INJURY EFFECT ON TUBE FORMATION BY CULTURED HUMAN VASCULAR ENTOTHELIAL CELLS. Cell Biol Toxicol 1995;11(1):29-36.

The effect of methylmercury chloride (MeHg) on growth and tube formation by cultured human umbilical vein endothelial cells (HUVECs) was investigated. HUVECs were collected by enzymatic digestion with collagenase. Precultivation of HUVECs with MeHg at concentrations of 1.0-50.0 mumol/L exerted negligible effects on the viable cell number, while the viable cell number was slightly reduced at 100 mumol/L and fell to zero at concentrations exceeding 500.0 mumol/L MeHg. The viable cell number was depressed in a concentration-dependent manner. Tube formation was studied by culturing the cells on gelled basement membrane matrix (Matrigel). Treatment of HUVECs with 0.15.0 mumol/L MeHg for 24 h inhibited tube formation dose-dependently. Fetal bovine serum (FBS) increased tube formation in a dose-dependent manner, with half-maximum stimulation of tube formation at approximately 3.4% FBS. The length of tube formation decreased time-dependently at concentrations of 0.1 and 1.0 mumol/L MeHg. Pretreatment of Matrigel with 1 mumol/L MeHg before the cell seeding reduced the tube formation by HUVECs. These results suggest that the growth and tube formation by HUVECs is susceptible to MeHg cytotoxicity, and that MeHg could be injurious to endothelial cell function.

325
Renault JY, Caillaud JM, Chevalier J. ULTRASTRUCTURAL CHARACTERIZATION OF NORMAL AND ABNORMAL CHONDROGENESIS IN MICROMASS RAT EMBRYO LIMB BUD CELL
CULTURES. Toxicol Appl Pharmacol 1995;130(2):
177-87.

Inhibition of chondrogenesis in limb bud cell micromass cultures has been proposed as a short-term teratogen detection test. Validation studies were performed by testing large series of reference compounds and comparing their teratogenic potential with their ability to inhibit chondrogenesis; however, there are few reports describing the histological and ultrastructural changes associated with inhibition of chondrogenesis in vitro. The objective of this study was to provide a qualitative description of the histological and ultrastructural alterations induced by three chondrogenesis inhibitors: retinoic acid (RA) and 6-aminonicotinamide (6AN), two teratogens, and doxylamine succinate (DS), a nonteratogen compound. In addition, in order to have a basis for the interpretation of the morphological alterations induced by the test compounds, the histological and ultrastructural changes which occur during the time course of chondrogenesis in control cultures were described and compared with those in rat embryo limb buds. We found that RA at 0.5 mug/ml led to a marked decrease in the number and size of cartilaginous foci; most cells lacked morphological signs of differentiation but their ability to proliferate was unaffected. At concentrations of 2 mug/ml and more,

6AN delayed cell proliferation, reduced staining of the extracellular matrix, and induced the formation of endoplasmic cisternae. DS at 50 mug/ml affected both differentiation and proliferation; pigment deposits were observed in chondrocytes, suggesting phospholipid metabolism disorders. In conclusion, this study showed that inhibition of chondrogenesis in this simple cell culture system can be associated with different types of histological and ultrastructural alterations. Examination of these alterations can provide useful information about the teratogenic potential of tested compounds and their mechanism of action.

326
Hooghe RJ, Ooms D. USE OF THE FLUORESCENCE-ACTIVITED CELL SORTER (FACS) FOR IN
VITRO ASSAYS OF DEVELOPMENTAL TOXICITY. Toxicol In Vitro 1995;9(3):349-54.

Our objective is to predict embryotoxicity with reliable in vitro techniques. In several exptl. systems, differentiation is accompanied by changes in the glycosylation pattern of cell-surface glycoconjugates. This is also the case with embryonal carcinoma cells. We have monitored the expression of receptors for wheat germ agglutinin (WGA). Murine embryonal carcinoma cells (P19 and F9) were exposed in vitro to xenobiotics for 1-3 days, then incubated successively with WGA-biotin (15 mug/mL) and streptavidin-phycoerythrin (SA-PE) (20 mug/mL), each for 30 min at room temp. Cell-surface fluorescence was then analyzed using a fluorescence-activated cell sorter (FACS). Exposure to 1 muM retinoic acid, a known inducer of differentiation, altered glycosylation as indicated by changes in WGA binding. Clear-cut effects were also obsd. after exposure to salts of arsenic (20 muM), or nickel (50 muM), and to methotrexate (1 mug/mL), fluorouracil (1.3 mug/mL) or actinomycin D (0.04 mug/mL). These compds. affected the percentage of pos. cells, the intensity of labeling, or both. Two non-teratogenic compds. (metronidazole and sulfonilamide) have also been tested and had no effect. Lectin histochem. of embryonal carcinoma cells exposed to potentially toxic agents holds promise as a method for predicting embryotoxicity. FACS anal. allows rapid quantification.

327
Andrews JE, Ebron-McCoy M, Nau H, Kavlock RJ. VALDIATION OF AN IN VITRO TERATOLOGY SYSTEM USING CHIRAL SUBSTANCES: STEREOSELECTIVE TERATOGENICITY OF
4-YN-VALPROIC ACID IN CULTURED MOUSE EMBRYOS. Toxicol Appl Pharmacol 1995;132(2):310-6.

In vitro systems are important for toxicity testing as well as for investigating the mechanism of action of xenobiotics. The validation of such in vitro systems is often incomplete and extrapolation to the in vivo situation is equivocal. In the present study, the authors studied the effects of enantiomers of an analog of the antiepileptic drug valproic acid (VPA):R(+)- and S(-)-4-yn-VPA (R- and S-2-n-propyl-4-pentynoic acid), which have previously been shown to induce selective teratogenicity in mice after in vivo administration, in mouse whole-embryo culture (WEC). Aq. solns. of the sodium salts of the pure R- and S-enantiomers as well as R,S-4-yn-VPA (racemic mixt.) or VPA itself were added to the culture medium at 0, 0.075, 0.15, 0.3,

0.6, or 1.2 mmol/L and embryos were evaluated 24 h later. The S-4-yn-VPA enantiomer induced clear concn.-dependent dysmorphogenesis that was evident even at the lowest concn. The primary anomalies were neural tube defects, erratic neural seams, blisters, and rotational defects. Embryolethality was obsd. at 1.2 mmol/L. The R-4-yn-VPA enantiomer was neither embryotoxic nor dysmorphogenic at any tested concn. The lack of biol. activity over 24 h in WEC with the R-enantiomer suggests also that, as previously shown in vivo, there was no racemization of this isomer to the more active S-enantiomer. The racemic mixt. of R and S isomers appeared to be slightly more embryolethal and dysmorphogenic than VPA. Overall, the potency of the S-enantiomer was approx. four times that of VPA. Therefore, the rank order of the four chems. tested was S(-) >> S(-), R(+) > VPA >>> R(+), which is in agreement with the effects obsd. in in vivo exposed mice. These data demonstrate a direct stereoselective effect of these compds. on the embryo. This is the first illustration of the stereoselectivity of a xenobiotic in the WEC in vitro test system. Pure and stable enantiomers, which induce stereoselective toxicity in vivo, are demonstrated to be valuable for validation of this in vitro system.

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Kishi J, Noda Y, Goto Y, Nakayama T, Nonogaki T, Mori T. ANALYSIS OF IN VITRO DEVELOPMENTAL BLOCK OF RAT EMBRYOS: ASSESSMENT FROM THE VIEW POINT OF OXYGEN
TOXICITY. J Reprod Dev 1994;40(4):285-91.

The authors have already reported that the block to development in cultured 1-cell rat embryos was overcome using HECM-1, a protein-free chem. defined medium without glucose or phosphate. In addn., the authors showed the beneficial effect of low oxygen tension in rat embryos. In this study, in order to clarify the reason why in vitro developmental block of rat embryos is overcome in HECM-1, the authors examd. the effects of phosphate, glucose, and L-cysteine on the in vitro development of rat embryos. Furthermore, the authors examd. their effects on the prodn. of H2O2 in embryos by the fluorometric method. The addn. of KH2PO4 even at only 1 muM completely inhibited the development of rat (Wistar) embryos either from the 1-cell stage or the 2-cell stage. The addn. of glucose at 1000 mug/mL significantly decreased the blastulation rate. The prodn. of H2O2 in embryos cultured under 5% O2 was significantly ($P<0.01$) lower than that of embryos cultured under 20% O2 in HECM-1. H2O2 prodn. in embryos cultured in mKRB was significantly ($P<0.01$) higher than that in embryos cultured in HECM-1, irresp. of the oxygen tension. The addn. of neither phosphate nor glucose to HECM-1 affected the H2O2 prodn. at any concn. examd. L-Cysteine significantly decreased the H2O2 prodn. in embryos in mKRB, but developmental block could not be overcome by the addn. of L-cysteine to mKRB. These results revealed that glucose and phosphate independently inhibited the development of rat embryos in vitro, and suggest that the mechanism of in vitro developmental block of rat embryos could not be explained simply in terms of oxygen toxicity.

329
Lane M, Gardner DK. REMOVAL OF EMBRYO-TOXIC AMMONIUM FROM THE CULTURE MEDIUM
BY IN SITU ENZYMATIC CONVERSION TO GLUTAMATE. J Exp Zool 1995;271(5):356-63.

An enzymatic method for removing embryo-toxic ammonium from culture medium has been developed. Ammonium, produced by both embryo metabolism and spontaneous breakdown of amino acids at 37~C, is transaminated by glutamate dehydrogenase to nontoxic glutamate. Initially, the individual components of the transamination reaction were titrated against mouse embryo development in vitro to determine embryo-safe levels. ADP, an allosteric activator of glutamate dehydrogenase, was found to inhibit embryo development and was therefore omitted from the final formulation (alpha-ketoglutarate, 0.44 mM; glutamate dehydrogenase, 0.375 U; NADH, 0.12 mM). It was found that 0.30 mM ammonium could be removed from the culture medium in situ in 3 h. In situ removal of ammonium significantly increases both blastocyst cell number, implantation, fetal development, and fetal weight after transfer. Removal of ammonium by the conventional method of renewing the culture medium also increased blastocyst cell number but did not affect postimplantation development. In conclusion, it is possible to alleviate the toxic effects of ammonium in vitro on pre- and postimplantation mouse embryo development by its transamination in situ, thereby facilitating the continual exposure to embryo-derived factor(s) which stimulates both pre- and postimplantation development.

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Pinski J, Schally AV, Yano T, Groot K, Srkalovic G, Serfozo P, Reissmann T, Bernd M, Deger W, et al. EVALUATION OF THE IN VITRO AND IN VIVO ACTIVITY OF THE L-, D,L- AND D-Cit6 FORMS OF THE LH-RH ANTAGONIST CETRORELIX (SB-75). Int J Pept Protein Res 1995;45(5):410-7.

The objective of this study was to examine the in vivo and in vitro gonadotropin-inhibiting potencies, edematogenic activities and the receptor binding affinities of the D-Cit6, and L-Cit6 forms of the LH-RH antagonist Cetrorelix. To demonstrate the suppressive effects of two different diastereomers of SB-75 and their racemic mixt. on LH and FSH release, [D-Cit6] SB-75 was injected s.c. in doses of 2.5 and 10 mug/rat, [DL-Cit6]-SB-75 in doses of 5 and 20 mug/rat and [L-Cit6]-SB-75 in doses of 12.5 and 50 mug/rat to castrated male rats. Two hours after administration, there was no difference in LH levels between rats injected with the L-form and control animals, indicating a low activity and(or) a rapid enzymic degrdn. of this peptide. The (1:1) diastereomeric mixt. was only about half as potent in suppression in LH release compared to [D-Cit6]-SB-75. Serum FSH levels were suppressed for more than 48 h after the administration of 10 mug [D-Cit6]-SB-75 and 20 mug of [DL-Cit6]-SB-75, resp. [D-Cit6]-SB-75 administered at a dose of 2 mug/rat induced 100% inhibition of ovulation, while 4 mug/rat of the DL-Cit6 peptide were necessary to produce the same effect. [L-Cit6]-SB-75 given at a high dose of 40 mug/rat produced only 14% inhibition of ovulation. The D-Cit6 form of SB-75 produced skin lesions with a much smaller diam. than the L-isomer, and was about 34 times less edematogenic. [D-Cit6]-SB-75 was bound more powerfully to high-affinity pituitary LH-RH receptors than either DL-Cit6 or L-Cit6 analogs. In vitro assays based on the superfusion of dispersed rat pituitary cells on a column, followed by RIA for LH, also demonstrated a lower inhibitory activity for the L-Cit6 analog, but the differences between D-, DL- and L-citrulline analogs

were smaller than in vivo. The results indicate that the LH-RH antagonist [D-Cit6]-SB-75 is more effective in suppression of gonadotropin release in vivo and in vitro, less edematogenic and possesses higher binding affinity to pituitary LH-RH receptors than the DL- and L-citrulline decapeptide analogs.

331
Hansen DK, Grafton TF. COMPARISON OF DEXAMETHASONE-INDUCED EMBRYOTOXICITY IN
VITRO IN MOUSE AND RAT EMBRYOS. Teratogenesis Carcinog Mutagen 1994;14(6):281-9.

Previous work demonstrated that rat embryos were more susceptible to the growth retardation effect of the synthetic glucocorticoid dexamethasone (DEX) in vivo than were mouse embryos. The purpose of this study was to examine this species difference using an in vitro system. Embryos of CD rats and CD-1 mice were cultured in a whole embryo culture system with concentrations of DEX from 5 to 250 mug/ml. Rat embryos were explanted on day 9 of gestation (GD 9: plug day = GD 0), while mouse embryos were removed on GD 8. After 48 h in culture, each viable embryo was evaluated for morphological score, and the number of somite pairs, crown-rump, and head lengths, as well as DNA and protein concentrations were determined. A reduced morphological score was observed for mouse embryos at 5 mug DEX/ml, but a significant decrease in this parameter was only observed at DEX concentrations of : 100 mug/ml in rat embryos. Significant reductions in the number of somite pairs were observed at 25 mug/ml for mouse embryos and 100 mug/ml for rat embryos. Crown-rump and head lengths as well as DNA and protein concentrations were significantly decreased at 100 mug/ml in mouse embryos and 150 mug/ml in rat embryos. Therefore, in vitro mouse embryos were adversely affected by lower concentrations of DEX than were rat embryos for each of the six end points examined in this study. This species sensitivity in vitro could be due to inherent genetic differences or to the slightly different developmental stages evaluated using the culture system. The species sensitivity observed in vivo may be due to a maternal difference in the pharmacodynamics of the drug.

332
Shibata M, Watanabe T. IN VITRO ASSESSMENT OF TERATOGENIC POTENTIAL OF CIS-1-[4-(P-MENTHANE-8-YLOXY) PHENYL] PIPERADINE. J Toxicol Sci 1995; 20(1):9-14.

The teratogenic potential of cis-1-[4-(p-menthane-8-yloxy) phenyl] piperadine (YM9429) was assessed by an in vitro culture method using fetal palatal tissues of CD rats. YM9429 induced fetal cleft palate in vivo in CD rats when dams were orally treated during days 11-14 of pregnancy at a dose of 500 mg/kg/day, but no abnormalities were detected when treated on days 15-16 (Shibata, 1993). After 4 successive days of oral treatment during days 11-14 of pregnancy, the maternal plasma levels of the drug were <5 mug/mL on day 15 and <1 mug/mL on day 16. After a single oral dosing on day 14, the fetal levels were .apprx.0.3 mug/g-fetus at 2 h postdosing. When the fetal maxillary regions removed from the normal fetuses on day 15 of pregnancy were cultured with YM9429 at concns. of 5 and 1 mug/mL-medium for 48 or 72 h, normal and full contact of the palatal shelves was obsd. These results supported the

previous findings that YM9429 demonstrated no teratogenic effects in vivo by maternal treatment on days 15-16 of pregnancy, and suggested other mechanisms than the direct influences on the fetal palatal shelves in the latter phases of morphogenesis including reorientation, horizontal extension, and adhesion.

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Daston GP, Baines D, Elmore E, Fitzgerald MP, Sharma S. EVALUATION OF CHICK EMBRYO NEURAL RETINA CELL CULTURE AS A SCREEN FOR DEVELOPMENTAL TOXICANTS.
Fundam Appl Toxicol 1995; 26(2):203-10.

This paper describes a study to evaluate the concordance with in vivo results of an in vitro screen for developmental toxicants. The screen is a primary culture of chick embryo neural retina cells (CERC) which undergo processes of cell-cell recognition and interaction, growth, and differentiation over a 7-day culture period. Each of these developmentally significant events is measured sep. as formation of multicellular aggregates, protein content, and glutamine synthetase activity, resp. A total of 45 chems., 24 of which have been shown to be teratogenic at some dosage to mammalian embryos in utero, 7 of which are embryotoxic (but not teratogenic) in utero a high dosage, and 14 of which have not produced developmental toxicity in vivo, were evaluated in this assay by investigators who were blinded to the identity of the chems. Chems. were tested up to concns. that were frankly cytolethal, or up to a max. of 4 mg/mL. Chems. were present only during the first 24 h of culture. The chems. were selected to be representative of a variety of chem. classes (e.g., solvents, metals, food additives, anticonvulsants, antineoplastics). In several cases, pairs of structurally similar compds. with different developmental toxic potencies (e.g., valproate and 2-en-valproate, formamide, and N,N-dimethylformamide) were tested. Of the 31 developmental toxicants, 25 affected at least one endpoint in the assay at concns. which are achievable in vivo (i.e., below the systemic concn. at a LD), yielding a false-neg. rate of 19%. Two of the nondevelopmental toxicants, saccharin, and penicillin G, had adverse effects at concns. below those that may be biol. achievable in vivo, giving a false-position rate of 14%. Overall concordance with in vivo results by these criteria was 82%. Quant. comparisons were also made between the lowest obsd. effect concn. (LOEC) in the assay and (i) lowest developmentally toxic dosage (mostly i.p.) reported in rats or mice in vivo and (ii) LOEC in rodent whole embryo culture. In the first instance, 77% of the LOECs (LOELs) were within an order of magnitude and 93% were within a factor of 30. In the second instance 81% of the LOECs were within an order of magnitude. Potency ranking of four alkoxy acids was comparable in CERC and in vivo rodent embryo. These results indicate that the CERC assay is concordant with developmental toxic potential and potency for the diverse group compds. selected, and that it could serve as a preliminary screen for developmental toxicity.

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Murakami M, Hosokawa S, Yamada T, Harakawa M, Ito M, Koyama Y, Kimura J, Yoshitake A, Yamada H. SPECIES-SPECIFIC MECHANISM IN RAT LEYDIG CELL TUMORIGENESIS BY PROCYMIDONE. Toxicol Appl Pharmacol 1995;131(2):

244-52.

To clarify the mechanism of species difference in the induction of testicular interstitial cell tumor (ICT, Leydig cell tumor) between rats and mice, male Sprague-Dawley rats and ICR mice were fed procymidone at dietary concentrations of 700, 2000 or 6000 ppm and 1000, 5000, or 10,000 ppm, respectively, for 3 months. The Leydig cell functions were evaluated by serum testosterone and luteinizing hormone (LH) levels, testosterone levels in the testis, LH levels in the pituitary, the capacity of the testis to respond to gonadotropin stimulation, i.e., the production of testosterone in vitro, and by the testicular binding of labeled human chorionic gonadotropin (hCG). Measurement of testosterone and LH levels in rat serum, the testis, or the pituitary showed that both hormones were enhanced throughout the 3-month treatment period. The hypergonadotropism was associated with the increase of interstitial cell response to hCG in vitro for up to 3 months. As with rats, both serum and pituitary LH were increased in mice at 4 weeks but not at 13 weeks. However, in contrast to rats, no significant increase in testosterone was observed in mice either in vivo or ex vivo during the course of the study. This suggests a difference between the rat and mouse in the response of the Leydig cell to the LH stimulation associated with procymidone administration. These differences in the response of interstitial cells to procymidone may be the basis for the distinct species responses to procymidone-induced Leydig cell tumorigenesis. The sustained response of the Leydig cells to stimulation in the rat results in chronic hyperplasia and subsequent benign tumor formation, while the attenuated response of Leydig cells in the mouse is associated with neither hyperplasia nor neoplasia.

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McMaster ME, Munkittrick KR, Jardine JJ, Robinson RD, Van Der Kraak GJ. PROTOCOL FOR MEASURING IN VITRO STEROID PRODUCTION BY FISH GONADAL TISSUE.
Can Tech Rep Fish Aquatic Sci 1995;1961:I-VIII,1-78.

336
Lazo JS, Kondo Y, Dellapiazza D, Michalska AE, Choo K HA, Pitt BR. ENHANCED SENSITIVITY TO OXIDATIVE STRESS IN CULTURED EMBRYONIC CELLS FROM TRANSGENIC
MICE DEFICIENT IN METALLOTHIONEIN I AND II GENES. J Biol Chem 1995;270(10):5506-10.

Embryonic cells from transgenic mice with targeted disruption of metallothionein I and II genes expressed no detectable metallothionein either constitutively or after treatment with cadmium, in contrast to cultured cells that were wild type or heterozygous for the loss of the metallothionein genes. Metallothionein null cells were most sensitive to the cytotoxic effects of cadmium, the membrane permeant oxidant tert-butylhydroperoxide, and the redox cycling toxin paraquat. No marked differences were seen among the wild type,

heterozygous, or metallothionein null cells in glutathione levels or in the activity of CuZn-superoxide dismutase, glutathione peroxidase, or catalase. Nevertheless, metallothionein null cells were more sensitive to

tertbutylhydroperoxide-induced oxidation as ascertained by confocal microscopic imaging of dichlorofluoroscein fluorescence. These results indicate basal metallothionein levels can function to regulate intracellular redox status in mammalian cells.

337
Seeley MR, Faustman EM. TOXICITY OF FOUR ALKYLATING AGENTS ON IN VITRO RAT EMBRYO DIFFERENTIATION AND DEVELOPMENT. Fundam Appl Toxicol 1995;26(1):136-42.

The relative developmental toxicity of four direct acting, alkylating agents was determined in primary cultures of differentiating rat embryo midbrain (CNS) and limb bud (LB) cells and compared with that observed in the rat whole embryo postimplantation culture system. The alkylating agents tested include methylnitrosourea (MNU), ethylnitrosourea (ENU), methyl methanesulfonate (MMS), and ethyl methanesulfonate (EMS). These alkylating agents have been shown to produce developmental toxicity following either in vitro or in vivo exposure. Viability for both CNS and LB was assessed by a neutral red dye assay. Differentiation of CNS cells was assessed by hematoxylin staining of neurons; differentiation of LB cells was assessed by Alcian blue staining of extracellular proteoglycans. Relative potencies of these compounds in the cell culture system were not the same as those observed in the embryo culture system. Whereas rank order of potency in the cell culture system, for viability and differentiation, was MMS > MNU > ENU > EMS, rank order in the embryo culture system, for embryo lethality and malformations, was MNU > ENU > MMS > EMS. Effective concentrations for cell culture viability and differentiation by MNU and ENU in cell culture were about three to nine times higher than comparable values previously reported for embryos, while effective concentrations for MMS and EMS were two to seven times lower than those observed in the embryos. Differences in potency between the two culture systems may be related to differences in formation and repair of DNA adducts, as well as differences in culture conditions.

338
Sandberg JA, Murphey LJ, Olsen GD. PHARMACOKINETICS AND METABOLISM OF COCAINE IN MATERNAL AND FETAL GUINEA PIGS. Neurotoxicology 1995; 16(1):169-78.

The pharmacokinetics and metabolism of cocaine (COC) were determined in late gestation maternal and fetal guinea pigs. After a single i.v. dose of 2-12 mg/kg, the average : SD total body clearance of COC was 59 : 16 ml/min/kg and was not dose dependent. However, volume of distribution was 2.1 and 3.9 l/kg, mean resident time (MRT) was 42 and 57 min, and elimination half-life was 34 and 49 min at the 2 and 4 mg/kg dose of COC, respectively. With the exception of an increased MRT, the pharmacokinetics were similar after s.c. COC administration. Benzoylecgonine (BE) and benzoylnorecgonine (BN) were major and persistent metabolites. Norcocaine (NOR) concentrations were low and transient. After chronic maternal administration of 6 mg/kg COC s.c., there was no difference between maternal and fetal plasma COC concentrations one hour after the last injection, but COC and BN accumulated in amniotic fluid. Examination of in vitro metabolism of COC in fetal and maternal guinea pig hepatic microsomes demonstrated minimal fetal N-demethylation and induction of

maternal N-demethylation by chronic COC exposure. The minimal fetal N-demethylation suggests BN seen previously in vivo after chronic maternal COC administration resulted from maternal formation of NOR and subsequent maternal and/or fetal hydrolysis to BN.

339
Srivastava SC, Kumar R, Prasad AK, Srivastava SP. EFFECT OF HEXACHLORO-CYCLOHEXANE (HCH) ON TESTICULAR PLASMA MEMBRANE OF RAT. Toxicol
Lett 1995;75(1-3):153-7.

The study deals with the analysis of residue of hexachlorocyclohexane (HCH) and its possible damaging potential on testicular plasma membrane of rats. In vitro studies were conducted by exposing plasma membrane of testis with 1.46 1.46ibition in the activity of the Ca2+-ATPase, Na+ + K+ + Mg2+-ATPase and 5' Nucleotidase. In vivo studies were carried out following repeated dermal exposure to HCH at a dose level of 50 or 100 mg/kg/day for 60 days to male rats. The results show significant decrease in the activities of 5'-Nucleotidase, Ca2+-ATPase, Na+ + K+-ATPase and Mg2+-ATPase in the plasma membrane of testis following exposure to HCH. The analysis of the residues of HCH reveals the presence of significant quantities of its different isomers viz., alpha, beta, gamma and delta in the testicular plasma membrane of rats given in vivo dermal exposure of this pesticide. These results suggest that the presence of HCH residue may be a factor in inhibiting the marker enzymes of the plasma membrane of testis.

340
Singh PB, Kime DE. IMPACT OF GAMMA-HEXACHLOROCYCLOHEXANE ON THE IN VITRO PRODUCTION OF STEROIDS FROM ENDOGENOUS AND EXOGENOUS PRECURSORS IN THE
SPERMIATING ROACH, RUTILUS RUTILUS. Aquatic Toxicol 1995;31(3): 231-40.

Testicular fragments from spermiating roach were incubated with 0, 1, 10 and 20 mg/L of gamma-hexachlorocyclohexane (gamma-HCH), and either carp hypophyseal homogenate (chh) or 3H-17-hydroxyprogesterone (3H-17P). The endogenous production of testosterone (T), 17-hydroxyprogesterone (17P), 17,20beta-dihydroxy-4-pregnen-3-one (17,20,betaP), 11-ketotestosterone (KT), testosterone glucuronide (TG), and 17,20,beta-dihydroxy-4-pregnen-3-one glucuronide (1 7,20betaPG) was measured by radioimmunoassay. of gamma-HCH on the testicular metabolism of 3H-17P was examined by thin layer and high performance liquid chromatography. Endogenous production of T was stimulated by 1 mg/L gamma-HCH but inhibited at higher doses, while its glucuronide was higher in all incubations containing gamma-HCH compared with controls. 17P and KT production was lower at all concentrations of gamma-HCH compared with controls, but the low endogenous 17,20betaP production was unaffected. gamma-HCH had a much lower impact o metabolism of exogenous 17P than on endogenous precursors. The major metabolite of 17P in the testis, 17,20betaP, showed a significant increase in yield in response to gamma-HCH together with a decrease in production of its glucuronide. There was also a significant increase in yield of testosterone at some concentrations, but none of the other metabolites were affected by gamma-HCH exposure. The results show that

the balance of steroid production by testes of spermiating roach is significantly perturbed by gamma-HCH and suggests that the pesticide affects predominantly the stages of steroidogenesis leading to 17-hydroxyprogesterone production.

341
Kemp RB. CYTOTOXICITY IN AN ANCHORAGE-INDEPENDENT FIBROBLAST CELL LINE MEASURED BY A COMBINATION OF FLUORESCENT DYES. Methods Mol Biol 1995;43:211-8.

342
Kristen U, Kappler R. THE POLLEN TUBE GROWTH TEST. Methods Mol Biol 1995; 43:189-98.

343
Knoll M, Shaoulian R, Magers T, Talbot P. CILIARY BEAT FREQUENCY OF HAMSTER OVIDUCTS IS DECREASED IN VITRO BY EXPOSURE TO SOLUTIONS OF MAINSTREAM AND
SIDESTREAM CIGARETTE SMOKE. Biol Reprod 1995;53(1):29-37.

Epidemiological data support a correlation between smoking and increased incidence of ectopic pregnancy, yet the causal mechanism responsible for this relationship is unknown. The purpose of this study was to examine the effect of solutions containing dissolved mainstream (MS) or sidestream (SS) cigarette smoke on ciliary beat frequencies (CBF) in explants of hamster oviducts. MS smoke is the puff inhaled by an active smoker, while SS smoke leaves the burning end of cigarette. SS smoke is inhaled by both active and passive smokers. Experiments were performed in handmade perfusion chambers using infundibula from hamster oviducts. After a short incubation in Earle's balanced salt solution containing HEPES buffer (EBSS-H), chambers were flushed with one of six types of smoke solution prepared in EBSS-H, and incubation continued 19 min. A second perfusion (washout) was then done using EBSS-H alone to determine whether effects induced by the smoke solutions could be reversed. CBF were determined at three times in both the smoke and washout solutions, and means were compared to values obtained in the initial EBSS-H incubation. All smoke solutions except the SS particulate solution inhibited CBF in a dose-dependent manner. Whole MS and whole SS smoke solution at the highest strength tested caused the greatest inhibition and in some cases completely stopped ciliary beating. Both single-strength and 0.1-strength MS gas phase solutions, which contained concentrations of nicotine in the range found in typical human smokers, produced about 50% inhibition of ciliary beating. Inhibition was generally seen within 2-12 min of adding smoke solutions. In all cases, washout of smoke solution caused an increase in CBF. In some washouts, CBF returned to initial values found in EBSS-H within 2 min of incubation in the recovery medium. However, in both MS and SS whole smoke solutions, CBF did not return to the initial EBSS-H values during the washout interval. In SS gas phase solutions, inhibition was not observed during exposure to the smoke solution but did occur during washout, suggesting that the mechanism of action of SS gas phase solutions differs from the other types of smoke solution tested. These results show that components in both MS and SS cigarette smoke can inhibit beating of oviductal cilia in vitro and that

inhibition can be at least partially reversed when the smoke solution is replaced by EBSS-H.

344
Hoyes KP, Johnson C, Johnston RE, Lendon RG, Hendry JH, Sharma HL, Morris ID. TESTICULAR TOXICITY OF THE TRANSFERRIN BINDING RADIONUCLIDE 114mIn IN ADULT AND NEONATAL RATS. Reprod Toxicol 1995;9(3):297-305.

Adult (70 d) and neonatal (7 d) male rats were dosed (i.p.) with 37 MBq/kg (1 mCi/kg; approximately 1 mug elemental indium/kg) 114mIn, a transferrin-binding radionuclide. In adults, approximately 0.25% of the injected activity localised within the testis by 48 h postinjection and remained constant for up to 63 d. In neonates, 0.06% of the activity was in the testis by 48 h, and this declined such that by 63 d only 0.03% remained. At 63 d, treated rats had reduced sperm head counts and abnormal testicular histology that was more marked in animals dosed as adults than as neonates. In vitro, uptake of 114mIn into seminiferous tubules isolated from 7-, 20-, or 70-d-old rats was compared with that of 125I. Both radionuclides were readily accumulated by the tubules. Whilst 114mIn uptake into 20- and 70-d tubules was inhibited by excess transferrin, uptake into 7-d tubules was unchanged. 125I uptake was not affected by excess transferrin. These data support the contention that some radionuclides may cross the blood-testis barrier by utilisation of the physiologic irontransferrin pathway, which may lead to greater testicular damage in adult compared to neonatal animals.

345
Genbacev O, Bass KE, Joslin RJ, Fisher SJ. MATERNAL SMOKING INHIBITS EARLY HUMAN CYTOTROPHOBLAST DIFFERENTIATION. Reprod Toxicol 1995;9(3):245-55.

Differentiation of the specialized epithelial cells of the placenta, termed cytotrophoblasts, is a particularly important aspect of placental development during the first trimester of pregnancy. During this process cytotrophoblast stem cells either fuse to form the syncytium or aggregate to form cell columns that adhere to, then invade the uterus. We found that chorionic villi from early gestation placentas of mothers who smoke showed a marked reduction in cell columns, a defect that could not be corrected by placing them in culture. We used two different in vitro models to determine if nicotine plays a role in the etiology of this defect. Exposing early gestation chorionic villi from nonsmoking women to nicotine inhibited subsequent cell column formation in vitro. Nicotine also inhibited normal first trimester cytotrophoblast invasion, apparently by reducing the ability of treated cells to synthesize and activate the 92 kDa type IV collagenase, an important mediator of invasion in vitro. These results suggest that maternal cigarette smoking inhibits the trophoblast differentiation pathway that leads to column formation and uterine invasion. This effect, which is due at least in part to the effects of nicotine, may contribute to the growth retardation observed in fetuses of mothers who smoke during pregnancy.

346
Jahan I, Bai L, Iijima M, Kondo T, Namba M. KARYOTYPIC ANALYSIS IN THE PROCESS OF IMMORTALIZATION OF HUMAN CELLS TREATED WITH 4-NITROQUINOLINE

1-OXIDE. Acta Med Okayama 1995;49(1):25-8.
Holladay SD, Smith BJ, Luster MI. B LYMPHOCYTE PRECURSOR CELLS REPRESENT SENSITIVE TARGETS OF T2 MYCOTOXIN EXPOSURE. Toxicol Appl Pharmacol 1995;131(2):309-15.

Exposure of experimental animals and humans to the Fusarium trichothecene metabolite, T2 toxin, has been associated with a variety of immunosuppressive effects, including altered parameters of humoral-mediated immunity. Although T2 toxin is cytotoxic in vitro to lymphocytic cells, limited information is presently available regarding the contribution of such a mechanism to immunosuppression in vivo, or to potential immune cell targets. In the present report, subchronic T2 toxin treatment of timed-pregnant B6C3F1 mice resulted in significant and selective depletion of fetal liver cells expressing low levels of surface CD44 and CD45 antigens, suggestive of possible lymphoid progenitor cell sensitivity to this agent. Evaluation of CD45R antigen expression in fetal liver supported such a hypothesis, demonstrating a significant reduction in fetal liver B lymphocytic cells in animals exposed to

T2 toxin. Subsequent in vitro T2 toxin exposure of fetal liver cells enriched for prolymphocytes by differential density gradient centrifugation demonstrated the presence of a highly sensitive subpopulation of cells that was eliminated in a selective, and near-complete, manner by T2 toxin exposure. This sensitive cell population was observed to have light-scatter characteristics of CD45R+ B-lineage lymphocytes. Additional studies in adult mice demonstrated a reduction in CD44lo and CD45R+ bone marrow cells similar to that seen in fetal liver, indicating that T2 toxin may also target immature B lymphocytes in this hematopoietic compartment. Taken together, these data suggest that the precursors of B cells may represent, for unknown reasons, highly sensitive targets of T2 toxin exposure.

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Fukuoka M, Kobayashi T, Hayakawa T. MECHANISM OF TESTICULAR ATROPHY INDUCED BY DI-N-BUTYL PHTHALATE IN RATS: VI. A POSSIBLE ORIGIN OF TESTICULAR IRON DEPLETION. Biol Pharm Bull 1994;17(12):1609-12.

In previous studies we have described mechanisms of testicular atrophy whereby di-n-butyl phthalate (DBP) caused a sloughing of the germ cells, prior to the testicular atrophy; this sloughing might be attributed to iron depletion in the blood and the testicular interstitial cells. To determine whether the iron depletion is mediated by iron-release from hemoglobin (Hb), the effects of DBP upon erythrocytes have been studied. In the in vivo studies, it was observed that DBP induced glutathione (GSH) depletion, a decrease in GSH reductase activity and Heinz body formation in the red blood cells, and iron release from Hb. In the in vitro studies, in which mono-n-butyl phthalate (MBP), a metabolite of DBP, was incubated with erythrocytes, Heinz bodies and iron release from Hb were observed. The present study proposes that a mechanism for the testicular atrophy induced by DBP might involve Heinz body formation, accompanied by iron release from Hb followed by depletion of iron in the blood and testes.

348
Gray LE, Klinefelter G, Kelce W, Laskey J, Ostby J, Ewing L. HAMSTER LEYDIG CELLS ARE LESS SENSITIVE TO ETHANE DIMETHANESULFONATE WHEN COMPARED TO RAT
LEYDIG CELLS BOTH IN VIVO AND IN VITRO. Toxicol Appl Pharmacol 1995;130(2):248-56.

It has been reported that ethane dimethanesulfonate (EDS) is a Leydig cell toxicant that affects rats and hamsters (Kerr et al., 1987), while, in contrast, the Leydig cells of mice are relatively insensitive to the toxicant. In the rat, there is a rapid decline in levels of testosterone (T) within hours after EDS administration. However, T production, spermiogenesis, and fertility are restored within a few weeks as new Leydig cells are formed from undifferentiated cells in the interstitium of the testis. In an earlier study, we found, as expected. that ejaculated sperm counts (ESCs) reached a nadir 10 days after adult rats were dosed with EDS at 65 mg/kg ip along with serum and testicular T, testis and seminal vesicle weights, and in vitro T production, while, in contrast, EDS at 65 mg/kg had no effect on these endpoints in the Syrian hamster (Gray et al., 1992). In the current study, when EDS was administered to 6, 12, and 18 month old hamsters at 100 mg/kg, it produced subtle effects on serum T and sex accessory gland weights, while dramatic effects were seen in similarly exposed rats. In addition, when testes were examined by light microscopy all treated rats displayed severely reduced Leydig cell numbers, while, in contrast, only one-third of the EDS-treated hamsters were affected, having moderately reduced Leydig cell numbers. In support of the histological data, 3beta-HSD enzyme activity was reduced by 99% of control in EDS-treated rats, but it was reduced by onl 35% of control in EDS-treated hamsters. An in vitro analysis of the effects of EDS on LH-stimulated T production by quartered testes demonstrated that the hamster testis was less sensitive to the direct effects of EDS than the rat testis. The IC50 after 3 hr in culture was greater than 1800 mug EDS/ml for the hamster quarter testes, while the IC50 for the rat quarter testes was 320 mug EDS/ml. In summary, these results demonstrate in vivo and in vitro that Leydig cells of hamsters are less sensitive to EDS than those of the adult rat.

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Eisses KT. DIFFERENCES IN TERATOGENIC AND TOXIC PROPERTIES OF ALCOHOL DEHYDROGENASE INHIBITORS PYRAZOLE AND 4-METHYLPYRAZOLE IN DROSOPHILA MELANOGASTER: II. ADH ALLOZYMES IN AN ISOGENIC BACKGROUND. Teratogenesis Carcinog Mutagen 1994;14(6):
291-302.

Pyrazole and 4-methylpyrazole (4-MP) are in vivo and in vitro inhibitors of alcohol dehydrogenase activity in mammals. The fruitfly Drosophila melanogaster has been used to demonstrate the influence of genetic variation in alcohol dehydrogenase alleles on the results of larval treatment with pyrazole and 4-MP. Genetic polymorphism of organisms involved in experiments with teratogenic and toxic agents is not often considered. Administration of pyrazole to larvae of isogenic D. melanogaster strains, differing mainly in their Adh alleles, caused large Notch-like teratogenic aberrations,

macrochaetae multiplication, and pupal mortality. The level of teratogenicity and developmental-toxicity of pyrazole was both concentration and Adh-genotype-dependent. The strain with the highest ADH activity showed smaller effects after the treatments with the two concentrations used. 4-MP does not cause morphological aberrations, although treatment of larvae with an isogenic background caused a high pupal mortality due to non-differentiated material in the pupal case.

350
Heaton MB, Bradley DM. ETHANOL INFLUENCES ON THE CHICK EMBRYO SPINAL CORD MOTOR SYSTEM: ANALYSES OF MOTONEURON CELL DEATH, MOTILITY, AND TARGET TROPHIC
FACTOR ACTIVITY AND IN VITOR ANALYSES OF NEUROTOXICITY AND TROPHIC FACTOR NEURO-PROTECTION. J Neurobiol 1995;26(1):47-61.

A series of in vivo and in vitro experiments were conducted to determine the influence of prenatally administered ethanol on several aspects of the developing chick embryo spinal cord motor system. Specifically, we examined: (1) the effect of chronic ethanol administration during the natural cell death period on spinal cord motoneuron numbers; (2) the influence of ethanol on ongoing embryonic motility; (3) the effect of ethanol exposure on neurotrophic activity in motoneuron target tissue (limb bud); and (4) the responsiveness of cultured spinal cord neurons to ethanol, and the potential of target-derived neurotrophic factors to ameliorate ethanol neurotoxicity. These studies revealed the following: Chronic prenatal ethanol exposure reduces the number of motoneurons present in the lateral motor column after the cell death period (embryonic day 12 (E12)). Ethanol tends to inhibit embryonic motility, particularly during the later stages viewed (E9-E11). Chronic ethanol exposure reduces the neurotrophic activity contained in target muscle tissue. Such diminished support could contribute to the observed motoneuron loss. Direct exposure of spinal cord neurons to ethanol decreases neuronal survival and process outgrowth in a dose-dependent manner, but the addition of target muscle extract to ethanol-containing cultures can ameliorate this ethanol neurotoxicity. These studies demonstrate ethanol toxicity in a population not previously viewed in this regard and suggest a mechanism that may be related to this cell loss (i.e., decreased neurotrophic support).

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Cosenza ME, Bidanset J. EFFECTS OF CHLORPYRIFOS ON NEURONAL DEVELOPMENT IN RAT EMBRYO MIDBRAIN MICROMASS CULTURES. Vet Hum Toxicol 1995;37(2):118-21.

352
Ciapetti G, Granchi D, Stea S, Cenni E, Schiavon P, Giuliani R, Pizzaferrato A. ASSESSMENT OF VIABILITY AND PROLIFERATION OF IN VIVO SILICONE-PRIMED LYMPHOCYTES AFTER IN VITRO RE-EXPOSURE TO SILICONE.
J Biomed Mater Res 1995;29(5):583-90.

The functional response of peripheral blood lymphocytes isolated from 22 patients with silicone gel-filled breast implants was assessed after in vitro re-exposure to silicone. Using cell culture test methods to quantify proliferation and viability and/or activation of lymphocyte microcultures,

i.e., the uptake of tritiated thymidine (3H-TdR uptake test) and the reduction of formazan salts (MTT assay), interesting data were obtained. Peripheral blood lymphocytes purified from patients wearing silicone gel-filled breast implants react in vitro to silicone showing a statistically significant increase of both proliferation and viability, while healthy subjects do not respond on in vitro exposure to silicone. Differences resulted even more statistically significant when patients were divided into two groups depending on the type of surgery they underwent: patients with breast augmentation for aesthetic reasons seem to have an increased responsiveness in vitro to silicone compared to patients who experienced a reconstructive surgery of the breast. Although they are still preliminary, being referred to a limited population, these results suggest that the lymphocytes of patients with silicone gel-filled breast implants could be sensitized in vivo toward silicone; the re-exposure of these cells to silicone leads to a higher functional response which could be looked for by using quantitative in vitro test methods.

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Canteros G, Rettori V, Franchi A, Genardo A, Cebral E , Faletti A, Gimeno M, McCann SM. ETHANOL INHIBITS LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) SECRETION BY BLOCKING THE RESPONSE OF LHRH NEURONAL TERMINALS TO NITRIC OXIDE.

Proc Natl Acad Sci USA 1995;92(8):3416-20.

It has previously been shown that alcohol can suppress reproduction in humans, monkeys, and small rodents by inhibiting release of luteinizing hormone (LH). The principal action is via suppression of the release of LH-releasing hormone (LHRH) both in vivo and in vitro. The present experiments were designed to determine the mechanism by which alcohol inhibits LHRH release. Previous research has indicated that the release of LHRH is controlled by nitric oxide (NO). The proposed pathway is via norepinephrine-induced release of NO from NO-ergic neurons, which then activates LHRH release. In the present experiments, we further evaluated the details of this mechanism in male rats by incubating medial basal hypothalamic (MBH) explants in vitro and examining the release of NO, prostaglandin E2 (PGE2), conversion of arachidonic acid to prostanoids, and production of cGMP. The results have provided further support for our theory of LHRH control. Norepinephrine increased the release of NO as measured by conversion of (14C)arginine to (14C)citrulline, and this increase was blocked by the a, receptor blocker prazosin. Furthermore, the release of LHRH induced by nitroprusside (NP), a donor of NO, is related to the activation of soluble guanylate cyclase by NO since NP increased cGMP release from MBHs and cGMP also released LHRH. Ethanol had no effect on the production of NO by MBH explants or the increased release of NO induced by norepinephrine. Therefore, it does not act at that step in the pathway. Ethanol also failed to affect the increase in cGMP induced by NP. On the other hand, as might be expected from previous experiments indicating that LHRH release was brought about by PGE2, NP increased the conversion of (14C)arachidonic acid to its metabolites, particularly PGE2. Ethanol completely blocked the release of LHRH induced by NP and the increase in PGE2 induced by NP. Therefore, the results support the theory that norepinephrine

acts to stimulate NO release from NO-ergic neurons. This NO diffuses to the LHRH terminals where it activates guanylate cyclase, leading to an increase in cGMP. At the same time, it also activates cyclooxygenase. The increase in cGMP increases intracellular free calcium, activating phospholipase A2 to provide arachidonic acid, the substrate for conversion by the activated cyclooxygenase to PGE2, which then activates the release of LHRH. Since alcohol inhibits the conversion of labeled arachidonic acid to PGE2, it must act either directly to inhibit cyclooxygenase or perhaps it may act by blocking the increase in intracellular free calcium induced by cGMP, which is crucial for activation of both phospholipase A2 and cyclooxygenase.

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Esterman AL, Rosenberg C, Brown T, Dancis J. THE EFFECT OF ZIDOVUDINE AND 2'3'-DIDEOXYINOSINE ON HUMAN TROPHOBLAST IN CULTURE. Pharmacol Toxicol 1995;76(1):89-92.

Trophoblast from term and first trimester placenta, maintained in culture, were exposed to 20 mumoles/l zidovudine or 2'3'dideoxyinosine. Several indices of function were measured and compared to control trophoblast in parallel culture. The results from individual placentas were examined by Student's t-test and cumulative results by ANOVA. Neither zidovudine or 2'3'-dideoxyinosine had statistically significant effects on the function of term trophoblast, following a 48 hr exposure to the drug as indicated by hCG secretion, protein synthesis and glucose consumption. In one of five placentas exposed to zidovudine, progesterone secretion was reduced as compared to its control but remained in the high range. Zidovudine had no significant effect on cultured trophoblast isolated from first trimester placenta even after prolonged exposure to the drug for eleven days. Both term and first trimester trophoblast in culture tolerate prolonged exposure to high concentrations of zidovudine or 2'3'-dideoxyinosine. Human trophoblast in culture provides a safe in vitro model for the screening of drugs intended for use during pregnancy.

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Cain L, Chatterjee S, Collins TJ. IN VITRO FOLLICULOGENESIS OF RAT PREANTRAL FOLLICLES. Endocrinology 1995;136(8):3369-77.

The impact of various gonadotropic hormones on the growth and development of secondary follicles from primordial and primary follicles obtained by enzymic dissocn. of the ovaries of immature 14-day-old rats was studied in vitro. The substratum-adherent culture technique developed for studying folliculogenesis in the current study permitted direct visualization of follicular growth on a day-to-day basis by avoiding the cumbersome process of fixing and sectioning follicles in culture. The cultures were maintained in a serum-free modified McCoy's medium is a humidified atm. contg. 5% CO2 at 37 C. Daily observation of the culture dishes under the phase contrast microscope revealed that the follicles grew and developed from primordial to primary and secondary follicular stages in the presence of FSH. Large antral follicles were able to secrete estradiol and progesterone into the medium, indicating that the follicles are not merely formed by cellular reorganization, but are physiol. functional competent units. The organized release of the oocyte with

accompanying corona radiata was made possible in some secondary follicles with large antral structures by introducing LH into the culture medium. However, introduction of hCG (which has the biol. properties of LH) into the cultures on day 1 resulted in follicular degeneration within 3-4 days of culture. Follicular organization was also disrupted when LH was introduced together with FSH into the medium on day 1 of culture. Primordial or primary follicles obtained from the ovaries could survive, but could not transform to secondary follicles in the absence of FSH. The results of these in vitro studies indicate, and therefore are in agreement with earlier in vivo studies, that FSH alone is essential for the progression of folliculogenesis to the preovulatory condition, and that LH is essential for the organized expulsion of the oocyte from a mature follicle. This technique, described in the current study, for producing physiol. functional secondary follicles in culture not only allows progress in folliculogenesis to be monitored very closely, but also serves as a model for studying the various intrinsic factors that may be involved in the successful development of dominant mature Graafian follicles that can finally ovulate. It also facilitates access to the growing follicle along with its oocyte, which can, therefore, be used as a powerful model to study the effects of various test substances in follicular development. Addnl., the oocyte may be exptl. manipulated and subjected to in vitro fertilization for producing animal species that could be used for research purposes, clin. trials, and restoring species that are on the brink of extinction.

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Bourget P, Roulot C, Fernandez H. [TRANSPLACENTAL PASSAGE AND FETOPLACENTAL METABOLISM OF DRUGS: STUDY DESIGN, THERAPEUTIC CONTRIBUTION AND IMPLICATIONS.]
Therapie 1994;49(6):481-97. (Fre)

Pregnancy is a specific dynamic state and the potential usefulness of caring for a fetal and/or adjacent disorder by treating the mother is now well established. Pregnant women being excluded from the investigational field of clin. trials, only few studies exist concerning evaluation of the pergestational metab. or transplacental transfer (TPT) of drugs. Questions are extensive and complex. Does TPT occur at a given gestational age (GA), in the context of a particular type of pathol., when a drug is administered by a certain dosage regimen and if this is the case, what is the rapidity of penetration of the products of conception by the drug (bearing in mind its phys.-chem. characteristics); need harmful adverse effects on the child be feared; is such penetration desirable, of no consequence or dangerous; does the possibility exist of accumulation in the placenta, fetal tissue or amniotic fluid; should such findings modify the therapeutic regimens of drugs given to expectant mothers. After dealing with the ethical and physiol. context in which such research is undertaken, the authors review methods for the study of TPT developed both in vitro and in vivo. The current review covers the period between 1972 and 1993. Exchange mechanisms are complicated and models developed in vitro only partially reflect the actual equil. which develop. These include: 1. the perfused cotyledon model, which while simple, elegant and inexpensive, offers only a localized and fixed view of pregnancy; 2. the necessary study, using microsomes, of placental metabolic capacity

(enzyme cartog.). In vivo study of TPT is based upon various multicompartmental pharmacokinetic models, some of which have been relatively validated in animals. The simplest indicator for the in vivo evaluation of TPT of a drug in the human species is detn. of a feto-maternal blood concns. ratio (usually performed at the time of sepn.). The usefulness and limitations of this parameter are controversial, and it would seem preferable to assoc. it with a kinetic profile of variations in blood concns. established in the mother. Any extrapolation of a single result to fetal and adjacent tissues must be done with the greatest caution.

357
Hoermann R, Poertl S, Liss I, Amir SM, Mann K. VARIATION IN THE THYROTROPIC ACTIVITY OF HUMAN CHORIONIC GONADOTROPIN IN CHINSES HAMSTER OVARY CELLS ARISES
FROM DIFFERENTIAL EXPRESSION OF THE HUMAN THYROTROPIN RECEPTOR AND MICROHETEROGENEITY OF THE HORMONE. J Clin Endocrinol Metab 1995;80(5):1605-10.

The role of hCG as a stimulator of the human thyroid has been a subject of controversy, because discrepant results have been obtained in different in vitro assays. To explain the variation obsd. in the thyroid response to hCG, the authors investigated the ability of hCG and that of its isoforms and glycosylation variants to inhibit [125I]bovine (b) TSH binding and stimulate adenylate cyclase in 2 clones HO09 and JP26, of Chinese hamster ovary cells stably transfected with the human TSH receptor (hTSHr). The two clones differed with respect to the no. of hTSHr expressed per cell (34,000 in JP09 and 2,000 in JP26 cells). Both responded extremely well to bTSH; the cAMP response to 0.001 IU/L bTSH was distinguishable from basal values. Interestingly, JP09 cells were readily stimulated by hCG (20-100 mg/L; 0.52-2.6.times.10-6 mol/L) to release cAMP, whereas JP26 cells showed little if any response. Also, cAMP stimulation produced by asialo-hCG was 12-fold in JP09 cells and only 4-fold in JP26 cells compared to 45- and 67-fold stimulations by bTSH, resp. Stimulation by asialo-hCG was approx. 30% that of bTSH in JP09 cells, but less than 6% in JP26 cells. When assessing the thyrotropic activity of the micro-heterogeneous isoforms of hCG, more alk. pI forms were more active than those of a more acidic pI regardless of whether they were derived from normal or molar pregnancy urine. Further studies with hCG, asialo-hCG, asialo-galacto-hCG, and deglycosylated hCG revealed that removal of sialic acid caused a marked increase in both its affinity for hTSHr and its cAMP-releasing potency, whereas removal of further carbohydrate, although it slightly enhanced receptor binding, was detrimental to adenylate cyclase activation. In conclusion, differences in hTSHr expression may cause a variation in the cAMP response to hCG or its glycosylation variants, as does the microheterogeneity of the hormone itself. These mechanisms may be responsible at least in part for the divergent responses of different cell types to hCG and render interpretation of the physiol. meaning of the data obtained in recombinant receptor systems difficult.

358
Ealey PA, Yateman ME, Sandhu R, Dattani MT, Hassan MK, Holt SJ, Marshall NJ.

THE DEVELOPMENT OF AN ELUTED STAIN BIOASSAY (ESTA) FOR HUMAN GROWTH HORMONE.
Growth Regul 1995;5(1):36-44.

The basic characteristics of MTT-formazan prodn. by both quiescent Nb2 cells and those activated by fetal calf serum or human growth hormone (hGH) are described. These characteristics are exploited for the development of an MTT-ESTA bioassay for purified prepns. of lactogens such as growth hormone. The resulting in vitro bioassay is sensitive and precise, with a detection limit of about 0.05 mU hGH/L (19 ng/L) and a within-assay imprecision of 2.5% in the presence of 0.3 mU hGH/L (114 ng/L). When utilizing quiescent Nb2 cells for bioassays, large magnitudes of response are obsd. The major component of the response is clearly derived from metabolic activation of the cells, rather than increased cell proliferation. The response was abolished by anti-human growth hormone. Delayed addn. of the latter demonstrated that the presence of the hormone is required for the entire 96 h of the recommended bioassay incubation period to obtain the max. response. At high doses, the dose-response relation reaches a prolonged plateau which covers 4 orders of magnitude of incremental hormone concns. A decline in response is obsd. at the highest dose tested, 106 mU hGH/L (385 mg/L). This auto-inhibition is consistent with recent reports of a redn. in response due to stoichiometric blockade of sequential receptor dimerization which is crucial for activation of both somatogenic and lactogenic receptors by hGH.

359
Yuan YY, Gu ZP, Shi QX, Qin GW, Xu RS, Cao L. [IN VITRO INHIBITION OF SPERMATOZOA FERTILIZATION ABILITY OF GUINEA PIG BY CELASTROL.] Yaoxue Xuebao 1995;30(5):331-5. (Chi)

The effects of celastrol (Cel), isolated from Tripterygium wilfordii, on guinea pig sperm forward motility (FM), capacitation (Cap), the acrosome reaction (AR) and sperm penetration assay (SPA) were assessed in vitro. Cel (5 mug.cntdot.mL-1) was found to inhibit these spermatozoal functions, and the inhibitions were proportional to the concns. of Cel used. The potency in inhibition of Cel on the fertilizing ability in guinea pig spermatozoa in vitro seems to follow the order: Cap > FM > SPA > AR. The inhibitory effect appeared to be reversible after washing away Cel if the duration of exposure of spermatozoa to Cel was shorter than 3 h. In a comparative study, the inhibitory effects of Cel on guinea pig sperm FM and AR were significantly stronger than those of gossypol acetic acid.

360
Mares A, DeBoever J, Stans G, Bosmans E, Kohen F. SYNTHESIS OF A NOVEL BIOTIN-ESTRADIOL CONJUGATE AND ITS USE FOR THE DEVELOPMENT OF A DIRECT, BROAD
RANGE ENZYME IMMUNOASSAY FOR PLASMA ESTRADIOL. J Immunol Methods 1995;183(2):211-9.

The synthesis and purifn. by high pressure liq. chromatog. of a novel biotinoyl-diaminodioxaoctane-estradiol conjugate is described. The conjugate was used for the development of an enzyme immunoassay (EIA) for the direct

detn. of estradiol in human plasma. This assay was characterized by a low limit of detection (77 pM) and a broad working range. Estradiol concns. ranging from 77 to 24300 pM, i.e. from the lower levels obsd. in the follicular phase of the normal menstrual cycle to the very high levels attained during hyperstimulation for in vitro fertilization, could be detd. directly in undiluted plasma samples. Intra-assay variation ranged from 3.6 to 10.9% and interassay variation from 6.1 to 12%. The results obtained by the present EIA correlate well with target values obtained by isotope-diln. gas chromatog.-mass spectrometry (r = 0.96) and with results obtained by a direct RIA (r = 0.97). The EIA requires no special app., is simple, fast and robust, and could be applied in any clin. lab.

361
Liu C, Litscher ES, Wassarman PM. TRANSGENIC MICE WITH REDUCED NUMBERS OF FUNCTIONAL SPERM RECEPTORS ON THEIR EGGS REPRODUCE NORMALLY. Mol Biol Cell
1995;6(5):577-85.

To initiate fertilization in mice, free-swimming sperm binds to mZP3, an .apprx.83-kDa glycoprotein present in the ovulated egg zona pellucida (ZP). MZP3 is located periodically along the filaments that constitute the ZP. Sperm recognize and bind to specific oligosaccharides linked to one or more of five Ser residues clustered in the carboxy-terminal one-third of the mZP3 polypeptide. When all five Ser residues are converted to nonhydroxy amino acids by site-directed mutagenesis of the mZP3 gene, an inactive form of mZP3, called mZP3[ser], is secreted by embryonal carcinoma cells stably transfected with the mutated gene. Here, seven independent transgenic mouse lines were established that harbor the mutated mZP3 gene. In all lines, the mutant gene is expressed by growing oocytes and mZP3[ser] is synthesized, secreted, and incorporated into the ZP. Purified mZP3[ser] prepd. from ovaries of transgenic mice, like mZP3[ser] from transfected embryonal carcinoma cells, is inactive in sperm binding assays in vitro. On the other hand, the presence of mZP3[ser] in the ZP does not significantly affect either the binding of sperm to ovulated eggs in vitro or the reprodn. of the mice, i.e., the transgenic mice are fertile, breed at normal intervals, and produce litters of normal sizes. These results indicate that the no. of functional sperm receptors in the ZP can be reduced by more than 50% without adversely affecting fertilization of eggs in vivo.

RESPIRATORY TOXICITY
362
Giri SN, Hollinger MA. EFFECT OF CADMIUM ON LUNG LYSOSOMAL ENZYMES IN VITRO. Arch Toxicol 1995;69(5):341-5.

Labilization of lysosomal enzymes is often associated with the general process of inflammation. The present study investigated the effect of the pneumotoxin cadmium on the release and activity of two lung lysosomal enzymes. Incubation of rat lung lysosomes with cadmium resulted in the release of beta-glucuronidase but not acid phosphatase. The failure to "release" acid phosphatase appears to be the result of a direct inhibitory effect of cadmium

on this enzyme. The K1 for cadmium was determined to be 26.3 muM. The differential effect of cadmium on these two lysosomal enzymes suggests that caution should be exercised in selecting the appropriate enzyme marker for assessing lysosomal fragility in the presence of this toxicant. Furthermore, the differential basal release rate of the two enzymes from lung lysosomes may reflect the cellular heterogeneity of the lung.

363
Balls M, Fentem J. IN VITRO TEST SYSTEMS AND RESPIRATORY TOXICOLOGY. In: Jenkins PG, et al., editors. International Programme on Chemical Safety IPCS Joint Series, 18. Respiratory Toxicology and Risk Assessment; International Symposium; 1992 Oct 6-9; Hanover, Germany. Stuttgart: Wissenschaftliche Verlagsgesellschaft MBH: 1994. p. 291-314.

364
Hirabayashi T, Ochiai H, Sakai S, Nakajima K, Terasawa K. INHIBITORY EFFECT OF FERULIC ACID AND ISOFERULIC ACID ON MURINE INTERLEUKIN-8 PRODUCTION IN RESPONSE TO INFLUENZA VIRUS INFECTIONS IN VITRO AND IN VIVO. Planta Med 1995;61(3):221-6.

We investigated the effect of ferulic acid (FA) and isoferulic acid (IFA), which are active components of the rhizoma of Cimicifuga species used frequently as anti-inflammatory drugs in Japanese Oriental medicines, on murine interleukin-8 (IL-8) production in response to influenza virus infections in vitro and in vivo by antibody-sandwich enzyme-linked immunosorbent assay. In the in vitro study, the murine macrophage cell line RAW 264.7 was infected with influenza virus at a dose of 10 plaque forming units (PFU)/cell and cultured in the presence or absence of drugs. Both FA and IFA reduced the IL-8 levels in the 20-h conditioned medium in comparison with control in a dose-dependent manner. The effect of IFA was greater than that of FA: IL-8 levels were reduced to 43% and 56% of the control in the presence of 100 micrograms/ml of IFA and FA, respectively. In the in vivo study, mice were infected with 1,000 PFU of virus and received daily oral administrations of Cimicifuga heracleifolia extract (5 mg/mouse/day), FA (0.5 mg/mouse/day), IFA (0.125 mg/mouse/day), or phosphate buffered saline. The three drugs showed a tendency to reduce IL-8 levels in bronchoalveolar lavage (BAL) obtained 2 days after infection. Moreover, both FA and IFA also significantly reduced the number of exuded neutrophils into BAL. However, the drug administrations did not affect the virus yields in BAL. These data suggest that FA and IFA are novel and potent inhibitors of murine IL-8 production and might act as one of the main components of anti-inflammatory rhizoma of Cimicifuga species.

365
Kier LD, Wagner LM, Wilson TV, Li AP, Short RD, Kennedy GL Jr. CYTOTOXICITY OF ETHYLENE OXIDE/PROPYLENE OXIDE COPOLYMERS IN CULTURED MAMMALIAN CELLS. Drug
Chem Toxicol 1995;18(1):29-41.

Cytotoxicity was measured in vitro for 8 ethylene oxide/propylene oxide copolymers (EO/PO copolymers) using lactate dehydrogenase release from cultured mammalian cells as the endpoint. Three cell types were used in these

assays: Chinese hamster ovary cell line (AS52), rat lung epithelial cell line (LEC), and freshly isolated rat alveolar macrophages (RAM). A range of cytotoxicity was seen with toxic effects observed from 20 to > 20,000 micrograms/ml. The same relative order of toxicities were observed for all 3 cell lines although RAM cells appeared to be somewhat more sensitive. The in vitro cytotoxicity, as measured by LDH release and microscopic observations of the cells, correlated poorly with the in vivo inhalation toxicity. The most lethal compounds following acute inhalation (UCON 50-HB-5100 and UCON 50-HB-2000) were among the least toxic in the in vitro cytotoxicity screen. Conversely the 2 compounds which were the most toxic in vitro (Pluronic 17 R1 and Pluronic L64) did not produce any unusual degree of toxicity in inhalation studies. The results of these experiments indicate that these in vitro mammalian cell assays will not be useful, at least for these classes of chemistry, in prediction of in vivo inhalation toxicity.

366
Fiala ES, Sodum RS, Hussain NS, Rivenson A, Dolan L. SECONDARY NITROALKANES: INDUCTION OF DNA REPAIR IN RAT HEPATOCYTES, ACTIVATION BY ARYL SULFOTRANSFERASE AND HEPATOCARCINOGENICITY OF 2-NITROBUTANE AND 3-NITROPENTANE
IN MALE F344 RATS. Toxicology 1995;99(1-2):89-97.

The secondary nitroalkanes, 2-nitropropane, 2-nitrobutane, 3-nitropentane, 2-nitroheptane, nitrocyclopentane and nitrocyclohexane, as well as the primary nitroalkanes, 1-nitropropane, 1-nitrobutane, 1-nitropentane and 1-nitroheptane, were examined for their ability to induce DNA repair in rat hepatocytes and to serve as substrates for activation by partially purified rat liver aryl sulfotransferase in vitro. All of the secondary, but none of the primary nitroalkanes examined, induced significant DNA repair in rat hepatocytes. Also, the nitronates of all of the secondary nitroalkanes, but none of the primary nitroalkanes, served as substrates for the aryl sulfotransferase-catalysed production of 8-aminoguanosine and 8-oxoguanosine from guanosine in vitro. In a carcinogenicity assay using male F344 rats, the secondary nitroalkanes, 2-nitrobutane and 3-nitropentane, produced a highly significant incidence of hepatocarcinoma with metastases to the lungs, whereas the primary nitroalkane, 1-nitrobutane, was not carcinogenic. While a low incidence of hepatocarcinoma was also produced by cyclopentanone oxime, the results were not statistically significant. Since the secondary nitroalkane, 2-nitropropane, in contrast to the primary nitroalkane, 1-nitropropane, was also previously shown to be hepatocarcinogenic in rats, it is probable that secondary nitroalkanes constitute a hitherto unrecognized class of chemical carcinogens.

367
Bianchi V. V79 CYTOTOXICITY TEST FOR MEMBRANE DAMAGE. Methods Mol Biol 1995;43:151-60.

368
Price RJ, Renwick AB, Wield PT, Beamand JA, Lake BG. TOXICITY OF 3-METHYLINDOLE, 1-NITRONAPHTHALENE AND PARAQUAT IN PRECISION-CUT RAT LUNG SLICES. Arch Toxicol 1995;69(6):405-9.

The toxicity of 3-methylindole, 1-nitronaphthalene and paraquat has been studied in precision-cut rat lung slice cultures. Lung slices were prepared from male Sprague-Dawley rats using an agarose gel instilling technique with a Krumdieck tissue slicer and cultured for 24 h in a dynamic organ culture system. Treatment of rat lung slices with 3-methylindole, 1-nitronaphthalene or paraquat produced concentration dependent decreases in lung slice protein synthesis and potassium content. EC50 values (concentration to produce a 50% inhibition) for protein synthesis were 0.024, 0.27 and 0.57 mM for paraquat, 1-nitronaphthalene and 3-methylindole, respectively. These results demonstrate that precision-cut lung slices are a useful in vitro model system for studying the pulmonary toxicity of xenobiotics. Lung slices offer the potential as a rapid in vitro screen for identifying pulmonary toxicants and to evaluate species differences in response.

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Boor PJ, Gotlieb AI, Joseph EC, Kerns WD, Roth RA, Tomaszewski KE.

CHEMICAL-INDUCED VASCULATURE INJURY. Summary of the symposium presented at the 32nd annual meeting of the Society of Toxicology, New Orleans, Louisiana, March 1993. Toxicol Appl Pharmacol 1995;132(2):177-95.

The cross-sectional structure of the vasculature is comparatively simple, comprising three layers-the intima, adjacent to the lumen, the media, and the adventitia. Notwithstanding this simplicity, the vessels are host to a variety of reactions to injury. Two cell types, endothelial cells of the intima and smooth muscle cells of the media, are principal targets of damage and repair. The endothelial cells of the intimal layer of the vessel wall present a macromolecular barrier and are important in maintaining vessel integrity. When the integrity is compromised by physical or chemical injury, endothelial cells play a key role in the repair processes. The use of single-cell wound models allows the mechanisms of damage and subsequent repair to be studied in depth. Repair processes can be observed using time-lapse photography and differences between cytoskeleton changes during repair and reendothelialization of small and large wounds can be discriminated. In rats treated with the plant toxin monocrotaline, pulmonary vascular injury occurs which manifests as thrombosis and remodeling with consequent progressive pulmonary hypertension. In vivo and in vitro studies of the mechanism of monocrotaline toxicity suggest that the endothelial cells are an important target. In vitro studies show monocrotaline to be directly cytotoxic; in cells that survive, there are functional changes to the endothelial cells, resulting in a decreased repair capability which may lead to the complex, progressive lung lesions that develop. The other target cells of the vasculature are the smooth muscle cells of the media. Ingestion of primary amines allylamine and beta-aminopropionitrile (beta-APN) results in chronic vasculotoxicity to the aorta and medium-sized arteries. For allylamine, subtle changes in smooth

muscle result in medial hypertrophy and subintimal proliferation. The changes are slow to occur, taking weeks or months of repeated treatment. For beta-APN, which is the active ingred of the toxic sweet pea Lathyrus odoratous, vascular toxicity is manifested by fatal rupture of aortic aneurysms. When these agents

are administered concomitantly, a synergistic acute smooth muscle necrosis occurs in large elastic arteries and degenerative changes are seen in muscular arteries. A change in the target for toxicity of allylamine toward the vasculature may be responsible for this synergistic toxic insult. Medial smooth muscle necrosis is also noteworthy after administration of certain pharmaceutical agents of diverse structure and pharmacological activity. These agents induce arteriopathies in dogs and rats, although at different sites. In dogs, the coronary arteries are susceptible, whereas in rats the mesenteric arteries are the principal sites of injury. Since these agents are diverse in structure and biochemical activity, the mechanism of toxicity of these agents appears to be related to their pharmacodynamic activity rather than to a direct effect. Studies on the relationship between vasodilatory and hypotensive properties of these agents and toxic response suggest the mechanism may be related to profound and exaggerated hemodynamic properties.

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Nakamura Y, Romberger DJ, Tate L, Ertle RF, Kawamoto M, Adachi Y, Mio T, Sisson JH, Spurzem JR, Rennard SI. CIGARETTE SMOKE INHIBITS LUNG FIBROBLAST PROLIFERATION AND CHEMOTAXIS. Am J Respir Crit Care Med 1995; 151(5):1497-503.

Cigarette smoking is the most clearly recognized cause of pulmonary emphysema. Since loss of lung tissue, which characterizes emphysema, represents a balance between injury and repair, we hypothesized that cigarette smoke might contribute to the development of emphysema by inhibiting fibroblast proliferation and migration. To evaluate this, we examined the effect of cigarette smoke extract (CSE) on the proliferation and migration of human lung fibroblasts in vitro. CSE inhibited fibroblast proliferation and migration at noncytotoxic concentrations. When CSE was treated to remove volatile components, it showed less inhibitory activity on fibroblast proliferation. Therefore, we also examined acrolein and acetaldehyde, which are volatile components of cigarette smoke. Micromolar concentrations of acrolein and millimolar concentrations of acetaldehyde induced significant inhibition of fibroblast proliferation. In contrast, removal of volatile components did not eliminate the inhibitory activity of CSE for fibroblast migration, although acetaldehyde and acrolein alone were also capable of inhibiting chemotaxis. Cigarette smoke-induced inhibition of fibroblast proliferation and migration may impair lung repair following lung injury, and may thus contribute to the development of pulmonary emphysema.

371
Cherpillod P, Amstad PA. BENZO(a)PYRENE-INDUCED MUTAGENESIS OF p53 HOT-SPOT CODONS 248 AND 249 IN HUMAN HEPATOCYTES. Mol Carcinog 1995;13(1):15-20.

Human tobacco-related cancers show a high frequency of G-to-T transversions in several mutation hot-spot regions of the p53 tumor suppressor gene, probably the result of specific mutagens in tobacco smoke, most notably benzo(a)pyrene. To gain insight into the mechanism of formation of these G-to-T transversions in tobacco-associated carcinogenesis, we studied the mutagenesis of p53 codons 247-250 by benzo(a)pyrene in human hepatocellular carcinoma cells by restriction fragment length polymorphism-polymerase chain reaction genotypic

analysis. Benzo(a)pyrene preferentially induced G-to-T transversion in the second and third positions of codon 248 and C-to-A transversion in the first position of codon 248. However, benzo(a)pyrene did not induce base-pair changes in codon 249, which is a mutational hot-spot in aflatoxin-related hepatocarcinogenesis, in which predominantly G-to-T transversion in the third position of codon 249 is observed. The benzo(a)pyrene-induced G-to-T transversion in the middle position of codon 248, in which arginine is changed into leucine, is frequently observed in tumors of the lung. The other two benzo(a)pyrene-induced base-pair changes in codon 248, namely the C-to-A transversion in the first position and G-to-T transversion in the third position, do not lead to a change in the amino-acid composition of the p53 protein. These mutations are silent and therefore are not selected in tumors. It follows that benzo(a)pyrene-induced. mutability on the DNA level in p53 codons 247-250 correlates well with the type of mutation found in tumors of the lung, Therefore, our results support the hypothesis that benzo(a)pyrene is the etiological agent in tobacco-related cancers.

372
Lee RP, Forkert P-G. PULMONARY CYP2E1 BIOACTIVATES 1,1-DICHLOROETHYLENE IN MALE AND FEMALE MICE. J Pharmacol Exp Ther 1995;273(1):561-7.

Pulmonary cytotoxicity induced by 1,1-dichloroethylene (DCE) has been linked to the generation of reactive intermediates through a cytochrome P450-dependent pathway. In the present studies, our objectives were to investigate and compare cytochrome P450 isozyme-selective bioactivation of DCE in vitro in the lungs of male and female mice. Our results showed that CYP2E1-dependent p-nitrophenol hydroxylation was significantly higher in microsomes from female (0.45 : 0.01 nmol/mg protein/min) than from male (0.38 : 0.02 nmol/mg protein/min) mice. Lung microsomes from male mice incubated in the presence of an NADPH-generating system and increasing amounts of DCE (5-20 mM) exhibited corresponding decreases in p-nitrophenol hydroxylase activity (19%-50%); however, greater decreases (26%-70%) were observed in lung microsomes from female mice incubated under the same conditions. In contrast, alterations in CYP2B1-dependent 7-pentoxyresorufin O-dealkylation and CYP1A1-dependent 7-ethoxyresorufin O-dealkylation were not detected in any microsomal preparation incubated with DCE. Reaction with an anti-CYP2E1 antibody abolished the inhibition of p-nitrophenol ydroxylation by DCE. Protein immunoblotting revealed significant decreases in the intensity of the bands of microsomal samples incubated previously with DCE; in contrast, alterations in heme content were not evoked by reaction with DCE. Our results have demonstrated that CYP2E1, and not CYP2B1 or CYP1A1, mediated the bioactivation of DCE. Furthermore, this bioactivation occurred to a greater extent in lung microsomes from female than from male mice, which suggests that females may be at slightly greater risk for DCE-induced pneumotoxicity.

373
Miller RC, Marino SA, Brenner DJ, Martin SG, Richards M, Randers-Pehrson G, Hall EJ. THE BIOLOGICAL EFFECTIVENESS OF RADON-PROGENY ALPHA PARTICLES: II. ONCOGENIC TRANSFORMATION AS A FUNCTION OF LINEAR ENERGY TRANSFER. Radiat Res
1995;142(1):54-60.

Epidemiological studies have established an association between exposure to radon and carcinoma of the lung. However, based on data for either lung cancer in uranium miners exposed to radon or bronchial epithelial carcinomas in Japanese A-bomb survivors, it has not been possible to assign estimates of risk of. lung cancer for the general population exposed to radon in their homes. Based on past success with the excellent quantitative properties of the C3H 10T1/2 in vitro oncogenic transformation assay system, the relative biological effectiveness (RBE) for radiation-induced transformation for charged particles of defined LET has been determined. As the LET of the radiation was increased, the rate of induction of oncogenic transformation increased and the RBEm approached 20. At higher LETs, RBE dropped precipitously. The rapid drop in effectiveness for et particles with LETs between 120 and 265 keV/mum implies a lower quality factor than the 20-25 currently considered appropriate when estimating lung cancer mortality.

374
Walles SA, Victorin K, Lundborg M. DNA DAMAGE IN LUNG CELLS IN VIVO AND IN VITRO BY 1,3-BUTADIENE AND NITROGEN DIOXIDE AND THEIR PHOTOCHEMICAL REACTION
PRODUCTS. Mut Res 1995;328(1):11-9.

A UV-irradiated mixture of 1,3-butadiene and nitrogen dioxide (NO2) was tested for its potency to induce DNA damage measured as single-strand breaks (SSB) in lungs of mice. Both gases were also tested separately. After 16 h exposure a UV-irradiated mixture of 40 ppm butadiene + 20 ppm NO2, but not 20 ppm butadiene + 10 ppm NO2 + UV, induced a significant increase in SSB as measured by the alkaline unwinding technique. There was no increase in the level of SSB using the alkaline elution technique during the same testing conditions. However, after 5 h exposure to 60 ppm butadiene + 30 ppm NO2 + UV both methods demonstrated a significant increase in SSB. Mice were also exposed to butadiene at 80 and 200 ppm for 16 h and at 500 ppm for 5 h. DNA damage was demonstrated in both liver and lung after 5 and 16 h (only at 200 ppm) of exposure using the unwinding technique. Using the alkaline elution assay, a significant increase in the level of SSB in lung and liver was found only after 5 h of exposure. When mice were exposed to 30 ppm NO2 for 16 h or 50 ppm for 5 h, a significant increase in SSB was found with the unwinding technique. Alveolar macrophages from mice were also exposed in vitro to the gas mixture and to butadiene and NO2 separately. In these experiments, the DNA damage was studied with the unwinding technique. A significant effect was demonstrated with 40 ppm butadiene + 20 ppm NO2 + UV. NO2 itself contributed to some extent to the increase. Reasons for the discrepancies between the unwinding and the alkaline elution techniques are discussed.

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Price RJ, Renwick AB, Beamand JA, Escalagon F, Wield PT, Walters DG, Lake BG. COMPARISON OF THE METABOLISM OF 7-ETHOXYCOUMARIN AND COUMARIN IN PRECISION-CUT
RAT LIVER AND LUNG SLICES. Food Chem Toxicol 1995;33(3):233-7.

The metabolism of 7-ethoxycoumarin and (3-14C)coumarin was compared in

precision-cut rat liver and lung slices. The lung slices were prepared using an agarose gel instilling technique enabling the production of tissue cylinders followed by lung slices employing a Krumdieck tissue slicer. Both 50 muM 7-ethoxycoumarin and 50 muM (3-14C)coumarin were metabolized by rat liver and lung slices. 7-Ethoxycoumarin was converted to 7-hydroxycoumarin (7-HC) which was conjugated with both D-glucuronic acid and sulfate. 7-HC sulfate was the major metabolite formed by both liver and lung slices. (3-14C)Coumarin was metabolized by rat liver and lung slices to both polar products and to metabolite(s) that bound covalently to tissue slice proteins. The polar products included unidentified metabolites and 3-hydroxylation pathway products, with only very small quantities of 7-HC being formed. These results demonstrate that precision-cut lung slices are a useful model in vitro system for studying the pulmonary metabolism of xenobiotics. Moreover, the precision-cut tissue slice technique may be employed for comparisons of hepatic and extrahepatic xenobiotic metabolism.

376
Stringer B, Imrich A, Kobzik L. FLOW CYTOMETRIC ASSAY OF LUNG MACROPHAGE UPTAKE OF ENVIRONMENTAL PARTICULATES. Cytometry 1995;20(1):23-32.

We sought to establish a quantitative method using flow cytometry to study

uptake of environmental particulates by alveolar macrophages (AMs). We used right angle light scatter (RAS) to measure uptake of titanium dioxide, quartz, and diesel particulates. After incubation with TiO2 in vitro, AMs showed dose-dependent increases in both cell-associated particles visualized by microscopy and RAS measured by flow cytometry (e.g., fold increase RAS at 4, 8, 16, 32, and 80 micrograms/ml, respectively, = 2 +/- 0.1, 4.0 +/- 0.5, 5.5 +/- 0.5, 9.1 +/- 2.5, 14.3 +/- 0.9; mean +/- SEM). Similar results were obtained with quartz and diesel particles. A strong correlation was observed between particle load per cell and AM RAS after uptake of fluorescent latex beads or fluorescent TiO2 (coated with BODIPY-BSA) (R2 = 0.984, 0.997, respectively). Using this technique, we found AM uptake of environmental particulates to be substantially greater than that of a panel of myelomonocytic and epithelial cell lines, consistent with their physiologic role in pulmonary defenses. RAS measurements have also identified both calcium-dependent and calcium-independent components in AM interactions with inert particles. Although this technique does not allow precise quantitation of particle number or mass per cell, flow cytometric analysis of relative increases in RAS is a useful tool to study AM interactions with a variety of environmental particulates.

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Spaggiari L, Alfieri R, Cattelani L, Bobbio A, Rusca M, Urbani S, Foletti G, Tecchio T, Carbognani P, Petronini P, et al. HAEMMACCEL, A KEY FOR LUNG PRESERVATION? Acta Biomed Ateneo Parmense 1995;65(3-4):147-55.

Several and different solutions have been used for lung preservation but, at present, fluids and solutions are quite alike. In the last years extracellular type solutions have been progressively tested in experimental researches and have shown a better protection vs intracellular one. In this research we have

studied the effect of a low-potassium solution normally used as plasma expander (Haemmaccel, HM) on isolated foetal human fibroblasts (WI-38). HM has been compared with Belzer solution (UWS) after 16 hrs incubation at 10 degrees C and low-potassium solutions with dextran 2% and 5% after 6 hrs and 16 hrs incubation at 10 degrees C. Wi-38 cells have been seeded at the density of 9x10(4)/cm2 onto plastic well plates. Cellular viability was measuring using the rate of protein synthesis through the incorporation of 3H leucine in growth medium during a 1 hr incubation at 37 degrees C. The results were expressed as nmol 3 H leu/mg of proteins/minute and presented as means +/- SD; the comparison has been performed by the one way variance analysis test. After 16 hrs incubation at 10 degrees C HM preserves WI-38 cells significantly better than UWS. Comparing HM with LPD 2% and 5% a significant difference both at 6 hrs and at 16 hrs was observed. Our preliminary in vitro results confirm that low-potassium solutions are less toxic on isolated lung cells than intracellular one. Polygelin contained in HM could be considered a suitable colloidal substance that determine a better preservation if compared with Low-Potassium Dextran solutions.

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Malkusch W, Rehn B, Bruch J. IN VITRO METHOD FOR MEDICAL RISK ASSESSMENT OF LASER FUMES. Opt Laser Technol 1995;27(1):39-43.

Laser processing of different materials may produce toxic fumes. In preventive occupational medicine it is necessary to evaluate valid hygienic stds. for work places. The basis for such hygienic stds. is the classification of laser fumes by their fibrogenic, emphysematous, immunol. or other harmful potencies in biol. assay systems. This paper is part of a European project on laser safety. The part in this project is the development of a method for the investigation of lung responses using in vitro cell assays. The appropriate laser fume samples will be supplied by other groups in this European project. In contrast to the cell assays usually used in risk assessment, the method is based on isolated target cells in the lung, such as alveolar macrophages. The test criteria are mediator release, surfactant reactions, release of reactive oxygen species and cell proliferation. As demonstrated in the lung response to other dusts (minerals, fibers etc.) these parameters are medically relevant factors in the pathogenic alveolar dust response. The paper gives basic information about the method using lung cell assays and the results of known substances, in comparison with a dust generated by laser processing.

STRUCTURE ACTIVITY
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Zhao F, Mayura K, Hutchinson RW, Lewis RP, Burghardt RC, Phillips TD. DEVELOPMENTAL TOXICITY AND STRUCTURE-ACTIVITY RELATIONSHIPS OF CHLOROPHENOLS
USING HUMAN EMBRYONIC PALATAL MESENCHYMAL CELLS. Toxicol Let 1995;78(1):35-42.

The chlorophenols (CPs) comprise a major class of widely distributed and frequently occurring environmental contaminants. Previous studies have demonstrated the adverse effects of CPs on embryonic and fetal development.

HEPM (human embryonic palatal mesenchymal) and MOT (mouse ovarian tumor) cell lines have been utilized in complementary bioassays for the detection of teratogens, but not the CPs. In this study, the objectives were 2-fold: (1) to det. if the HEPM assay could be used to complement other bioassay systems of

nonhuman origin, i.e., Hydra attenuata (HA) and rat whole embryo culture (WEC), in the evaluation of the developmental toxicity of CPs, and (2) to delineate the ability of the HEPM assay to evaluate structure-activity relationships of pentachlorophenol (C5P), 2,3,4,5- tetrachlorophenol (C4P), 2,3,5-trichlorophenol (C3P), 3,5-dichlorophenol (C2P), 4-monochlorophenol (CP), phenol, and CP derivs. (i.e., acetates, sodium phenates and anisoles). HEPM cells were seeded into each well of a 24-well plate and cultivated for 24 h. The medium was replaced with fresh medium contg. various concns. of test chems. dissolved in DMSO (0.1%). After culturing for 72 h, the medium was removed, cells were trypsinized, and cell no. detd. The HEPM cell growth inhibition assay demonstrated a linear relation between the IC50 values of the CPs and degree of chlorine substitution. The IC50 values of C5P, C4P, C3P, C2P, CP, and phenol were 18.8, 21.5, 27.5, 63.0, 150.0 and 470.0 muM, resp. A clear structure-activity relation was obsd. between toxicity of CPs and the degree of chlorine substitution. The rank order of CP toxicity from the HEPM assay (i.e., C5P > C4P > C3P > C2P > CP > phenol) is in excellent agreement with previous in vitro and in vivo studies. However, contrary to published reports, the HEPM assay predicted that all CPs were teratogenic (false positives). These findings suggest that the HEPM cell growth inhibition bioassay may be useful to discriminate between subtle differences in structure-activity and, in combination with other bioassays, might facilitate the rapid detection and prioritization of diverse cytotoxins, including various developmental toxicants. Importantly, conclusions about the teratogenicity of a test chem. (via HEPM testing) should be approached with caution and confirmed with other teratogen-sensitive systems.

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Stehmann C, De Waard MA. RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY OF TRIAZOLE FUNGICIDES AGAINST BOTRYTIS CINEREA. Pestic Sci 1995;44(2):183-95.

The inhibitory activity of commercial and experimental triazole fungicides on the target enzyme, sterol 14alpha-demethylase (P45014DM), was studied in a cell-free sterol synthesis assay of Botrytis cinerea Pers. ex Fr. In order to assess structure-activity relationships, the inhibitory activities of the compounds on radial growth of the fungus were tested as well. The EC50 values (concentrations of fungicide inhibiting radial growth of B. cinerea on PDA by 50%) of all triazoles tested ranged between 10-8 and 10-5 M. IC50 values (concentrations of fungicides inhibiting incorporation of (2-14C)mevalonate into C4-desmethyl sterols by 50%) generally ranged between 10-9 and 10-7 M and correlated with inhibition of radial mycelial growth. However, differences in IC50 values did not reflect quantitatively the observed differences in EC50 values, since the ratio between EC50 and IC50 increased with decreasing fungitoxicity. For a limited number of compounds the correlation between intrinsic inhibitory activity and fungitoxicity was low. Both in-vitro tests were used to investigate structure-activity relationships for stereoisomers of

cyproconazole, SSF-109 and tebuconazole. Fungitoxicity and the potency to inhibit cell-free C4-desmethyl sterol synthesis correlated for all stereoisomers tested. Mixtures of isomers of tebuconazole or cyproconazole were slightly less active than the most potent isomer. The high activity of several commercial triazoles in both experiments implies that poor field performance of triazole fungicides against B. cinerea is due neither to insensitivity of the P45014DM nor to low in-vitro sensitivity of the fungus.

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Luker KE, Tyler AN, Marshall GR, Goldman WE. TRACHEAL CYTOTOXIN STRUCTURAL REQUIREMENTS FOR RESPIRATORY EPITHELIAL DAMAGE IN PERTUSSIS. Mol Microbiol 1995;16(4):733-43.

The respiratory epithelial pathol. of pertussis (whooping cough) can be reproduced by tracheal cytotoxin (TCT), a disaccharide-tetrapeptide released by Bordetella pertussis. TCT is a muramyl peptide, a class of peptidoglycan-derived compds. which have many biol. activities including adjuvanticity, somnogenicity, pyrogenicity, and cytotoxicity. The structural requirements for muramyl peptides to produce some of these biol. effects have been partially characterized. Using in vitro assays with respiratory epithelial cells and tissue, we have previously detd. that the disaccharide moiety of TCT is not involved in toxicity and that the side-chain functional groups of diaminopimelic acid (A2pm) are crucial for toxicity. In this study, we det. the importance of every amino acid, functional group and chiral center in the peptide portion of TCT. Although lactyl tetrapeptides are the most toxic of the TCT fragments, producing dose-response curves identical to TCT, the smallest analogs of TCT which are active in our assay are of the form X-gamma-(D)-Glu-meso-A2pm, where X may be an amino acid or a blocking group. Within this active substructure, main-chain chirality and all functional groups are essential for toxicity. This definition of the core region of TCT indicates that the TCT interaction site is unlike almost all other muramyl peptide interaction sites for which structure-activity data are available.