研究業績

論文等

化学・生化学

Leaf extract of *Wasabia* Japonica relieved oxidative stress induced by *Helicobacter pylori* infection and stress loading in Mongolian gerbils

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Bioscience, Biotechnology, and Biochemistry, 2010; **74(6)**: 1194-1199

Infection with *Helicobacter pylori* (*H. pylori*) can induce gastric disorders, and though its presence cannot explain disease pathogenesis and does not have associations with other factors, it is well known that *H. pylori* infection causes stomach inflammation following oxidative stress. We examined the suppressive effects of a leaf extract of *Wasabia japonica* on *H. pylori* infection and on stress loading in Mongolian gerbils. Following oral administration of wasabi extract of 50 and 200 mg/kg B.W./d for 10 d, the animals were exposed to restraint stress for 90 and 270 min. As for the results, the level of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in the stomach and oxidative DNA damage in peripheral erythrocytes at 270 min significantly increased. That elevation was significantly suppressed by the addition of the leaf extract. We concluded that the simultaneous loading of *H. pylori* infection and physical stress loading might induce oxidative DNA damage additively, while a leaf extract attenuated this DNA damage in the stomach as well as the peripheral erythrocytes.

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免疫毒性学

病態発現と副作用　3.免疫異常

大沢基保

医薬品トキシコロジー(改訂第4版), 南江堂, 東京 (2010) pp. 105-112

実験動物学

動物実験の標準化

高島宏昌

実験動物学の原理(Principles of Laboratory Animal Science翻訳), 学窓社, 東京(2011) pp.93-100

微生物学的標準化

高島宏昌

実験動物学の原理(Principles of Laboratory Animal Science翻訳), 学窓社, 東京(2011) pp.133-152

一般毒性学

Influences of *in vitro* tubule-like structures by two types of ultrafine titanium dioxide and zinc oxide

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Nano Biomedicine, 2010; **2(1)**: 52-58

We evaluated the *in vitro* influences of two types of ultrafine titanium dioxide differing in surface treatment and ultrafine zinc oxide on the area rate and length of tubule-like structures using a human angiogenesis kit. In addition, the influences of titanium and zinc oxide ions were evaluated. A comparison of influences on tubule-like structures between the two surface treatment methods of ultrafine titanium dioxide showed only slight influences of surface treatment providing water-repellency. Using ultrafine zinc oxide, no tubule-like structure formation was observed. The area rate of tubule-like structures was 84.0% for titanium ions and 78.3% for zinc ions at a concentration of 2.5 ppm, but this rapidly decreased with an increase in the ion concentration. These results may differ from those obtained in the nanoparticle dispersion state. It is possible that *in vivo* biological influences also differ between aggregation and dispersion states. Further studies based on the *in vivo* dispersion state are necessary.

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Effects of *in vitro* new capillary formation by C60 fullerene

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Nano Biomedicine, 2010; **2(2)**: 123-129

To examine the effects of C60 fullerene on angiogenesis, we investigated the effects of C60 fullerene on capillary regeneration from cells in the early stage of tubule-like structures using a human angiogenesis kit (Kurabo, Tokyo). In addition, using 8-Hydroxydeoxyguanosine (8-OHdG), we investigated its effects as an antioxidant to inhibit oxidative stress. The area ration and length of new blood vessels in the 5mg/mL group were 57.4 and 54.3%, respectively, compared with the control group. At 2.5 and 5 mg/mL, no significant difference was noted compared with the control group. The amounts of 8-OHdG in the 2.5 and 5 mg/mL groups were 89.4 and 87.5%, respectively, on comparison with the control group. Thus, no significant difference was noted. C60 fullerene was not sufficiently dispersed in the medium. Thus, C60 fullerene particles should be homogeneously dispersed in media. In the future, our results should be compared with those from dissolution using Polyvinylpyrrolidone (PVP) to clarify biological evaluation of new capillary formation.

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細胞毒性学

A study on the dose setting of test chemicals for the promotion assay in Bhas 42 cell transformation assay

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Alternative to Animal Testing and Experimentation, 2010; **15(1)**: 6-13

We have proposed a cell transformation assay using the Bhas 42 cells (Bhas 42 CTA) as an *in*

vitro method for predicting the carcinogenicity of chemicals. The Bhas 42 CTA consists of two assays: one is the initiation assay and the other is the promotion assay. An in-house study on Bhas 42 CTA had been performed using approximately a hundred test chemicals. In that study, seven chemicals induced the severe cell killing in the promotion assays and their promoting activities were unable to be evaluated. The aim of this study was to find the cause of severe cell killing that occasionally occurred in the promotion assay. We presumed that the severe cell killing was attributed to the failure of dose setting caused by the difference of treatment periods between the cell growth assay (for 3 days) and the promotion assay (for 10 days). In this study, we compared the inhibition rates in the cell growth assays between the chemical treatments for 3 days and 10 days. For seven chemicals that had induced the severe cell killing in the promotion assays, a larger inhibition was caused by the treatment for 10 days than for 3 days. For the chemicals whose promotion assays had succeeded, the growth inhibition was similar between two treatment conditions. These results demonstrated that the severe cell killing in the promotion assays was attributed to the failure of dose setting arising from the difference of the period of chemical treatment between the cell growth assay for dose setting and the promotion assay.

A Bhas 42 cell transformation assay on 98 chemicals: The characteristics and performance for the prediction of chemical carcinogenicity

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Mutation Research, 2010; **702(1)**:100-122

The Bhas 42 cell transformation assay is a short-term system using a clone of the BALB/c 3T3 cells transfected with an oncogenic murine ras gene (v-Ha-*ras*). The assay has previously been reported to be capable of detecting the tumor-initiating and tumor-promoting activities of chemical carcinogens according to the different protocols, an initiation assay and a promotion assay, respectively. We applied this short-term assay to 98 chemicals to characterize the assay and evaluate its performance for the detection of chemical carcinogenicity. When the assay results were compared with the existing genotoxicity data, the Bhas 42 cell transformation assay could detect a considerable number of Ames-negative and Ames-discordant carcinogens: and the promotion assay detected most of those Ames-negative and -discordant carcinogens. This fact suggested that the Bhas 42 cells behaved as initiated cells in the transformation assay. The performance indices were calculated from the assay results of 52 carcinogens and 37 non-carcinogens. The concordance was 78%, sensitivity 73%, specificity 84%, positive predictivity 86%, negative predictivity 69%, false negative 27% and false positive 16%. Of these values, the concordance, specificity, negative predictivity and false positive were superior and the other performance indices were equivalent to those of conventional genotoxicity tests. From overall results, we concluded that the accuracy of prediction of chemical carcinogenicity would be improved by introducing the Bhas 42 cell transformation assay into the battery of *in vitro* assays.

遺伝毒性学

Evaluation of a liver micronucleus assay in young rats (IV): A study using a double-dosing/single-sampling method by the Collaborative Study Group for the Micronucleus Test (CSGMT)/Japanese Environmental Mutagen Society (JEMS)-Mammalian Mutagenicity Study Group (MMS)

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Mutation Research, 2010; **698(1-2)**: 24-29

A collaborative study was conducted to evaluate whether a liver micronucleus assay using four-week-old male F344 rats can be used to detect genotoxic rat hepatocarcinogens using double-dosing with a single-sampling 4 days after the second dose. The assay methods were thoroughly validated by the seven laboratories involved in the study. Seven chemicals, 2,4-diaminotoluene, diethyl nitrosamine, *p*-dimethylaminoazobenzene, 1,2-dimethylhydrazine dihydrochloride, 2,4-dinitrotolunene, 2,6-dinitrotoluene and mitomycin C, known to produce positive responses in the single-dosing/triple-sampling method were selected for use in the present study, and each chemical was examined in two laboratories with the exception of 2,4-dinitrotolunene. Although several of the compounds were examined at lower doses for reasons of toxicity than in the single-dosing/triple-sampling method, all chemicals tested in the present study induced micronuclei in liver cells indicating a positive result. These findings suggest that the liver micronucleus assay can be used in young rats to detect genotoxic rat hepatocarcinogens using a double-dosing/single-sampling procedure. Further, the number of animals used in the liver micronucleus assay can be reduced by one-third to a half by using the double-dosing/single-sampling method. This reduction in animal numbers also has significant savings in time and resource for liver perfusion and hepatocyte isolation.

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Evaluation of a liver micronucleus assay in young rats (III): A study using nine hepatotoxicants by the Collaborative Study Group for the Micronucleus Test (CSGMT)/Japanese Environmental Mutagen Society (JEMS)-Mammalian Mutagenicity Study Group (MMS)

Hironao TAKASAWA[1], Hiroshi SUZUKI[2], Izumi OGAWA[3], Yasushi SHIMADA[4], Kazuo KOBAYASHI[5], Yukari TERASHIMA[5], Hirotaka MATSUMOTO, Chinami ARUGA[6], Keiyu OSHIDA[7], Ryo OHTA, Tadashi IMAMURA[2], Atsushi MIYAZAKI[2], Masayoshi KAWABATA[1], Shigenori MINOWA[1], Makoto HAYASHI[8]

Mutation Research, 2010; **698(1-2)**: 30-37

We have been investigating a liver micronucleus assay to detect genotoxic chemicals using young rats for several years, and had established its advantages with respect to using autonomous proliferation of young rat hepatocytes. Nine chemicals known to induce hepatotoxic effects such as necrosis (2,6-dinitrotolune, bromobenzene, isoniazid, phenacetin, allyl alcohol and thioacetamide), cholestasis (chlorpromazine hydrochloride and *α*-naphthyl isothiocyanate) and oxidative stress (clofibrate) were selected for this study. A liver micronucleus assay was conducted in 4-week-old male F344 rats using two or three dose levels of test chemicals given orally by gavage to evaluate the compound's ability to induce micronucleated hepatocytes. Several of these test chemicals were additionally examined in a peripheral blood micronucleus assay conducted concurrently and in the same animals. The genotoxic rodent hepatocarcinogen, 2,6-dinitrotoluene showed a positive result in the liver micronucleus assay, but the nongenotoxic hepatocarcinogens, clofibrate and thioacetamide

gave negative responses. Bromobenzene, known to produce DNA adducts but is noncarcinogenic in rodent liver, was judged equivocal in this assay. *α*-Naphthyl isothiocyanate is noncarcinogenic and showed negative response in the liver. The other four chemicals, known to be either noncarcinogenic or carcinogenic in other non-liver target organs, showed negative results in the liver micronucleus assay. Based on the results in the present study and previous report described above, it was concluded that this technique is able to effectively predict genotoxic rodent hepatocarcinogenicity, and does not give false positives due to hepatotoxicity.

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Induction effect of coadministration of soybean isoflavones and sodium nitrite on DNA damage in mouse stomach

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Food and Chemical Toxicology, 2010; **48(10)**: 2585-2591

We have already found that nitrite-treated isoflavones exhibit genotoxic activities toward *Salmonella typhimurium* TA 100 and 98 strains (submitted: nitrite-treated genistein). However, we have not demonstrated genotoxic activity induced by simultaneous treatment with isoflavones and $NaNO_2$ *in vivo*. In the present study, we examined whether coadministration of isoflavones (such as daidzein and genistein) and $NaNO_2$ induces DNA damage in the stomach of ICR male mice. Mice were coadministered with isoflavones (1mg/kg body weight) and $NaNO_2$ (10mg/kg body weight), and dissected to collect tissues at 1, 3, and 6h after administration. We used comet assay combined with repair enzyme formamidopyrimidine-*N*-glycosylase (FPG) to detect FPG-sensitive sites. An HPLC-ECD system was employed to determine 8-oxo-2'-deoxyguanosine (8-oxodG) in the stomach. In addition, we observed leukocyte infiltration by histopathological investigation, and measured total superoxide dismutase (SOD) in the stomach. We confirmed that oxidative DNA damage in the stomach was significantly increased by coadministration. Total SOD activities were also significantly stimulated by coadministration. However, the induction of inflammation in the stomach was not found. These data suggest that coadministration of isoflavones and $NaNO_2$ can cause DNA damage in the stomach because of the formation of radicals.

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環境衛生学

Basic research on developing scallop tissue reference material for quality assurance of diarrhetic shellfish poisoning (DSP) mouse bioassay (MBA): -Free fatty acid (FFA) in homogenized frozen scallop slurry and its effect on MBA-

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Journal of Environmental Chemistry, 2011; **21(1)**: 75-78

Diarrhetic shellfish poisoning (DSP) is one of the gastrointestinal illness caused by the consumption of

shellfish contaminated with toxigenic dinoflagellates. The main toxins responsible for DSP are Okadaic acid（OA）and its derivatives. Remarkable increase of free fatty acid（FFA）in the hepatopancreas（HP）of scallops during storage in a freezer is occasionally observed and it results in pseudo-positive with the MBA for DSP. In the process of making reference material（RM）for MBA, which is considered of a set of a vial containing a piece of filter infused with OA and DSP negative slurry of homogenized scallop whole meat（WH）, we investigated the concentration of FFA. The determination of OA and FFA concentrations was performed using liquid chromatography with a fluorometric detector for anthryl diazomethane（ADAM）derivatives. In this study FFA composition and toxicity were surveyed in homogenized scallop tissue stored in a freezer at -70° C for 4 months. Most of the samples were nontoxic as determined by mouse bioassay and showed low FFA concentration; one sample showed both toxic and high FFA concentrations. These results suggest that the determination of FFA concentration in scallop tissue by HPLC coupled with the MBA for DSP is important for RM.

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食品衛生学

食品安全性辞典　第2版

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共立出版, 東京（2010）

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デオキシニバレノール・ニバレノールの外部精度管理調査

笠間菊子, 小熊恭代, 福光　徹, 鈴木達也, 渡辺卓穂, 大島赴夫, 中島　隆[1]

食品衛生研究, 2010; **60(11)**: 25-34

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残留農薬検査の食品衛生外部精度管理調査

渡辺卓穂, 勝村利恵子, 高坂典子, 福光　徹, 鈴木達也, 大島赴夫

食品衛生研究, 2010; **60(12)**: 17-23

食品機能学

EL4腫瘍細胞に対するヤマブシタケ・マイタケの増殖抑制効果について

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日本補完代替医療学会誌, 2010; **7(1)**: 11-16

本研究はヤマブシタケおよびマイタケを飼料に添加しマウスに経口投与させることにより，EL4 腫瘍細胞の増殖を抑制するか否かを検討したものである．その結果，ヤマブシタケ単独添加およびマイタケ単独添加でも EL4 腫瘍細胞増殖抑制の傾向がみられた．また．フローサイトメトリーによって免疫担当細胞について検討したところ，マイタケの単独添加では，腫瘍細胞移植による脾臓でのキラーT細胞・NK細胞の減少を抑制した．ヤマブシタケ添加では腫瘍抑制の効果が得られたものの，マイタケ添加とは異なる免疫能の応答を示した．マイタケ80%，ヤマブシタケ20%を混合し，飼料に1%添加した群ではマイタケ，ヤマブシタケ単独よりも強い腫瘍細胞増殖抑制効果を示した．また，脾臓でのNK細胞・キラーT細胞の減少抑制効果がマイタケ単独添加と同様に認められた．

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発生神経毒性学

Sex-specific effects of early neonatal progesterone treatment on dopamine and serotonin metabolism in rat striatum and frontal cortex

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Life Sciences, 2010; **87(23-26)**: 738-742

Aims: The early neonatal period is critical for the development of the rodent brain. Neurosteroid levels in the brain decline from the late gestation to the neonatal period. Previous studies indicate effects of neurosteroid treatment during the neonatal period on the development of the dopaminergic system. In this study, we investigated the sex-specific effects of neonatal treatment with the neurosteroid progesterone on monoamine metabolism. Separately, we examined the contribution of pre-pubertal castration on the effect of neonatal treatment of pregnenolone (a neurosteroid precursor).

Main methods: Progesterone (Experiment 1) or pregnenolone (Experiment 2) treatments in Sprague-Dawley rats were performed from postnatal days 3 through 7. Castration in experiment 2 was performed in male rats at postnatal day 21. We measured the brain tissue contents of dopamine, serotonin (5-HT), and their metabolites in rats at age 10 weeks.

Key findings: Results showed that neonatal progesterone treatment altered striatal 5-hydroxy-3-indolacetic acid/5-HT ratios in males and females in opposite directions, in addition to dopaminergic effects. The treatment also influenced dopamine and 5-HT metabolism without sex-specificity in the frontal cortex. In addition, there was no significant difference in striatal monoamine metabolism between sham-operated, castrated and castrated pregnenolone-treated group.

Significans: The present result indicates a sex-specific influence of progesterone during the early neonatal period on the development of the serotonergic system, depending on brain region in addition to of the dopaminergic system.

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学会発表等

免疫毒性学

マウスの経口食物アレルギーモデルの発症機序: 腸間リンパ組織のT細胞サブポピュレーションの解析

新藤智子, 香取輝美, 金澤由基子[1], 大沢基保, 小島幸一, 手島玲子[2]

第17回日本免疫毒性学会学術大会　2010.9.9〜9.10(つくば)
同会講演要旨集, p. 120

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食物アレルゲン性の*in vitro*評価系の開発 (2)*In vitro*消化蛋白質の評価

香取輝美, 新藤智子, 大沢基保, 小島幸一, 手島玲子[1]

第17回日本免疫毒性学会学術大会　2010.9.9〜9.10(つくば)
同会講演要旨集, p. 135

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実験動物学

雌Hatano高回避系（HAA）および低回避系（LAA）ラットの社会性・不安・学習行動に関する研究

堀井康行[1,2], 川口真以子[3], 太田　亮, 槇原弘子[4], 嶋田　努[4], 油田正樹[4], 渡辺　元[1,2], 氷見敏行[3], 田谷一善[1,2]
第150回日本獣医学会学術集会　2010.9.16～9.18（帯広）
同会web page, I-13

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Stress reactive strain（High-avoidance rats）had shorter life-span than did non-reactive strain（Low-avoidance rats）

Ryo OHTA, Fumiaki KUMAGAI, Kenji USUMI, Makiko KUWAGATA, Noriko SAKURAI, Hideki MARUMO, Yoshiaki SAITO
The XVIIIth International Workshop on Genetic Systems in the Rat　2010.11.30～12.3（京都）
同会Program & Abstracts, p. 87

毒性病理学

食用油投与によるミニブタの病理組織学的変化

斉藤義明, 臼見憲司, 古谷真美, 立花滋博, 内藤由紀子, 永田伴子, 宮澤大介[1], 安井裕子[1], 山田和代[1], 大原直樹[1], 奥山治美[1]
第27回日本毒性病理学会　2011.1.27～1.28（大阪）
同会講演要旨集, p. 106

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一般毒性学

食品添加物及び食品汚染物質等の安全性評価試験

小野　宏
平成22年食品添加物研修会　2010.9.24（東京）

毛細血管の形成に及ぼすC60フラーレンの影響について（*in vitro*）

今井弘一[1], 亘理文夫[2], 高島宏昌, 西川哲成[3], 田中昭男[3], 武田昭二[1]
第3回ナノ・バイオメディカル学会大会　2010.9.17（横浜）
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生殖・発生毒性学

N,N'-ジフェニル-*p*-フェニレンジアミン（DPPD）により引き起こされた，ラット妊娠期間延長に関する検討

高島宏昌, 瀬沼美華, 桑形麻樹子, 古川　賢[1], 古谷真美, 吉田由香, 丸茂秀樹, 小島幸一, 今井弘一[2]
第50回日本先天異常学会学術集会　2010.7.8～7.10（淡路）
同会要旨集, p. 78

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In vitro 発生毒性試験法における3種類のES細胞による心筋への分化の比較について

今井弘一[1], 亘理文夫[2], 高島宏昌, 武田昭二[1]
第56回日本歯科理工学会学術講演会　2010.10.9～10.11(岐阜)
日本歯科理工学会誌, 2010; **29(5)**: 459
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8種類の歯科用合金組成金属元素イオンによる *in vitro* 血管新生の影響

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第8回日本再生歯科医学会学術大会　2010.10.29～10.30(名古屋)
同会講演集, p. 42
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細胞毒性学

A Bhas 42 cell transformation assay sensitive to Ames-negative and Ames-discordant carcinogens: Its performance for the prediction of chemical carcinogenicity

Ayako SAKAI, Kiyoshi SASAKI, Dai MURAMATSU, Shoko ARAI, Nobuko ENDOU, Sachiko KURODA, Kumiko HAYASHI, Yeon-mi LIM, Shojiro YAMAZAKI, Makoto UMEDA, Noriho TANAKA
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同会 web site: #4365

過酸化水素による形質転換細胞の選択を利用したBhas 42細胞形質転換試験の開発

佐々木澄志, 村松　大, 新井晶子, 遠藤伸子, 酒井綾子, 山崎晶次郎, 梅田　誠, 田中憲穂
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Validation work with BALB/c 3T3 and Bhas 42 cell transformation assays

Noriho TANAKA, Ayako SAKAI, Kiyoshi SASAKI
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96ウェル培養プレートを用いたBhas 42細胞形質転換試験の国際バリデーション研究(第I相)に関する報告

新井晶子, 佐々木澄志, 酒井綾子, 村松　大, 遠藤伸子, 水橋福太郎[1], 永井美穂[1], 浅倉眞澄[2], 田代信彦[3], 石井奈々[3], 鈴木雅也[1], 田中憲穂
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Bhas 42細胞を用いる形質転換試験による多層カーボンナノチューブの*in vitro*発がん性の検討

浅田　晋, 斉藤義明, 山影康次, 本間正充[1]

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多層カーボンナノチューブ(MWCNT)のCHL/IU細胞を用いた染色体異常試験

高橋俊孝, 浅田　晋, 原　巧, 豊泉友康, 斉藤義明, 熊谷文明, 山影康次, 本間正充[1]

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Bhas 42細胞を用いる形質転換試験のガイドライン化並びにハイスループット化

佐々木澄志, 村松　大, 新井晶子, 遠藤伸子, 黒田幸子, 林　久美子, 酒井綾子, 山崎晶次郎, Yeon-mi LIM, 梅田　誠, 和田昌憲[1], 田中憲穂

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酒井綾子, 佐々木澄志, 林　久美子, 村松　大, 新井晶子, 遠藤伸子, 黒田幸子, 水橋福太郎[1], 笠本佐和子[1], 永井美穂[1], 浅倉眞澄[2], 弘瀬秀樹[3], 石井奈々[3], Kamala PANT[4], Shannon W. BRUCE[4], Jamie E. SLY[4], Albrecht POTH[5], Susanne BOHNENBERGER[5], Thorsten KUNKELMANN[5], 山崎晶次郎, 梅田　誠, 田中憲穂

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An international validation study on a Bhas 42 cell transformation assay using 6-well plates for the prediction of chemical carcinogenicity

Ayako SAKAI, Kiyoshi SASAKI, Kumiko HAYASHI, Dai MURAMATSU, Shoko ARAI, Nobuko ENDOU, Sachiko KURODA, Fukutaro MIZUHASHI[1], Sawako KASAMOTO[1], Miho NAGAI[1], Masumi ASAKURA[2], Hideki HIROSE[3], Nana ISHII[3], Kamala PANT[4], Shannon W. BRUCE[4], Jamie E. SLY[4], Albrecht POTH[5], Susanne BOHNENBERGER[5], Thorsten KUNKELMANN[5], Shojiro YAMAZAKI, Makoto UMEDA, Noriho TANAKA

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遺伝毒性学

アミノ酸含有物質のための改良Ames試験(Treat & Wash法)の検討

川上久美子, 須井　哉, 桜井徳子, 奥富弘子, 太田　亮
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ヘアレスマウスを用いた*in vivo*皮膚コメットアッセイの検討

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Nasal instillation of nanoparticle-rich diesel exhaust particle affects emotional behavior and learning capability in rats

Syunji YOKOTA, Hiromasa TAKASHIMA, Takashi MIYAHARA, Yuka YOSHIDA, Tsukasa NEGURA, Yoshiaki SAITO, Naoyuki HIRABAYASHI, Takaho WATANABE, Ryo OHTA, Shinji HORIUCHI, Yuji FUJITANI[1], Seishiro HIRANO[1], Hidekazu FUJIMAKI[1]
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食品衛生学

小麦中Don-3-glucosideの分析法の検討

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特定原材料検査に関する外部精度管理の実施に向けた検討
笠間菊子, 小熊恭代, 鈴木達也, 穐山　浩[1], 大島赴夫, 小島幸一
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食品機能学

植物油摂取によってラットで認められる有害効果
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ミニブタにおけるカノーラ油の長期摂取の影響
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カノーラ油摂取による脳卒中易発高血圧自然発症ラットの病態生理学的変化
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発生神経毒性学

ラット胎生期バルプロ酸曝露の出生児脳発達への影響
桑形麻樹子, 小川哲郎[1,2], 塩田清二[1], 永田伴子
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Unraveling the effects of development on the olfactory system in a BrdU-induced developmental disorder model rat
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