Proceedings of the Fifteenth Conference about Laboratory Animals

held at

Tišnov (Czech Republic), May 23 – 25, 2012

The 15th Conference about Laboratory Animals organized by the Czech Laboratory Animal Science Association (SVLZ) was held at Tišnov, South Moravia, May 23 – 25, 2012. Most papers were devoted to laboratory animal protection and welfare, several presentations reported new data from experiments both in animals and alternative models.

NEW LABORATORY ANIMALS ACT

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There seems to be little disagreement on the importance of laboratory animals for modern medicine and pharmacology. Consequently, a new separate law on laboratory animals logically becomes a crucial legal matter to consider. One of the main arguments for an entirely new law is the Directive 2010/63/EU on the protection of animals used for scientific purpose which is intended to fully replace the current Directive 86/609/EHS. The new directive practically eliminates the sphere of agriculture from the topic and therefore we believe that the authority in chargé of the issue should rest no longer with the Ministry of Agriculture, but with the Ministry of Education, Youth and Sports which is responsible for science and research. Another argument for the new law is the fact that the current directive dates back to 1986 and our act on protection of animals against abuse to 1992, both legal norms being outdated. Moreover, based on the recommendation by the National Council for Economy and the Council for Research, Development and Innovation the Czech Republic Government plans to define and specify the priorities for our science and research and allocate substantial funding to them. One of the first practical steps to be taken is a draft of the intent and purpose of the new law to be duly submitted and enforced. This draft should be based on the current Act no. 246/1992 Coll., in particular its paragraphs 15-18f, and on the Directive 2010/63/EU on the protection of animals used for scientific purposes. It is also advisable to consider the Austrian Act no. 501/1989 Coll., on experimental animals and the currently arising Civil code which no longer define live animals as „things“.

EFFECT OF ENVIRONMENTAL ENRICHMENT AND GUINEA PIGS

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Guinea pigs are in our location breed not only as laboratory animals but of course as pets. Its reaction and habituation are determined by mother, keeping, contact intensity with keeper or animals and with a lot of other factors and aspects. Some of these aspects enriching the environment of animals kept in normal living environment. Adjustments for experimental breed animals can be hay, materials for building the nest and others (Sharmann 1991). In laboratories kept animal could suffer from deprivation and animal loss normal behavior, which evoke stereotypical behavior and passivity of animals (Wemelsfelder 1990). In short-term experiment (30 days) we observed activity of guinea pigs and its reaction on better keeping (time with keeper and grooming) and enriched environment with used hay, enriched food and enriched cages with toys. In experiment were 20 guinea pigs from 5 litters. Animals were divided in 2 groups, in each box were 2 animals. One group (10 guinea pigs) had improved care, enriched environment and spent more time with keepers, the other group was in boxes with normal environment without enriched environment, without more care. The aim of study was to discover, how the animals will be active, stressed and how the enrichment improves its cooperation. The guinea pigs with enriched environment and more time spent with keepers were significant more active than the animal from second group. The animals from boxes with enriched environment spent also significant more time with food, „playing“ with „toys“ and interact more after coming the keepers. They cooperated more after touching and were not so much stressed, as the other animals from non enriched group.

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ENRICHMENT AND EFFECT ON YOUNG PIGS AFTER WEANING

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Pigs are used not only for meat production as farm animals, pest or laboratory animals. Its behaviour is determined by mother (before weaning), keeping, contact among animals and to keepers and with many other factors. One of important factor is enriching the environment of animals kept in normal living environment. As additions for experimental or farm breed pigs can help hay or other materials for building the nest, toys, more intensive contact with keepers and others. Scientific studies of enrichment for pigs housed in indoor systems have focused on several types of ‘toys’, including objects such as tyres, chains, rubber hoses or dog toys (Schaefer *et al.* 1990, Apple and Craig 1992, Pearce and Paterson 1993, Pedersen *et al.* 1993, Feddes and Fraser 1994, Blackshaw *et al.* 1997, Hill *et al.* 1998). In short-term (30 days) experiment was observed activity of piglets (after weaning) and its reaction on better care (time with keeper, ...) and enriched environment with used toys (little ball, tires, football balls, shoes, high boots). In experiment were 40 pigs. Animals were divided into 2 groups, in each stall were 10 animals. First group (20 pigs in 2 stalls) had better keeping, enriched environment (toys) and spend more time with keepers, the other group was in boxes without enriched environment, with normal care. The aim of study was to evaluate, how the animals will be active and how the enrichment improve its cooperation and reduce stress. Piglets groups in enriched environment were significant more active to comparison to the animal from second (non enriched space). The animals with enriched environment spent more time playing toys and interact more after coming the keepers. The noises were for these pigs not the stressing factor. They cooperated more after touch and were not stressed so much as the other animals from no enriched group after coming the keeper in their fields.

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EFFECTS OF CHOSEN DRUGS ON BONE METABOLISM IN RATS

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Antihypertensive drugs are wide used agents for the treatment of hypertension but there the detailed information about their effects on the bone metabolism is missing. Therefore, the aim of the study was to investigate the effects of chosen antihypertensive drugs – amlodipine (calcium channel blocker) and metoprolol (selective β_1 receptor blocker without intrinsic sympathomimetic activity) on bone metabolism in healthy rats. 24 male albino Wistar rats (240±10 g) were pretreated by amlodipine and metoprolol (0.2 ml/100 g BW; gavage) a day for eight

weeks. Rats were divided into three groups of eight animals. 1. control group was treated with aqua pro injectione, 2. metoprolol was pretreated (MET; 5 mg/kg BW) and 3. group amlodipine (AML; 3 mg/kg BW) as suspension. We evaluated marker of bone resorption carboxy-terminal telopeptide of collagen I (ICTP), and markers of bone formation – osteocalcin (OC), amino-terminal propeptide of procollagen I (PINP) and bone alkaline phosphatase (BALP) using enzyme immunoassay method (ELISA). Bone mineral density (BMD) was measured with dual energy X-ray absorptiometry. Amlodipine pretreatment decreased concentration of ICTP to 77 %, 77 % ($p<0.05$), and BALP to 3 %, 5 % ($p<0.001$) of both control group and metoprolol pretreated animals, respectively. Amlodipine also decreased concentration of PINP to 63 % ($p<0.05$) of controls. Metoprolol pretreatment increased concentration of OC to 144 % ($p<0.05$) in comparison to control rats. Neither amlodipine nor metoprolol change BMD. Present results suggest that metoprolol may increase activity of osteoblasts and thus formation of bone matrix. Our data show that amlodipine decrease bone turnover in healthy male albino Wistar rats.

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EFFECTS OF METOPROLOL ON THE BONE METABOLISM IN RATS

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Antihypertensive drugs are wide used agents for the treatment of hypertension. Beta-blockers have been postulated to affect bone metabolism but the detailed information is missing. Therefore, the purpose of our study was to evaluate the effects of metoprolol (selective β_1 receptor blocker without intrinsic sympathomimetic activity) on bone metabolism in male albino Wistar rats. Adult rats (240±10 g; n=8) were divided into two groups. 1. control group was administered *aqua pro injectione* (0.2 ml/100 g BW; gavage), 2. metoprolol group with administration of metoprolol (0.5 ml in 0.2 ml *aqua pro inj.*/100 g BW; gavage) as a suspension daily for 8 weeks. Bone turnover markers were evaluated in serum: carboxy-terminal telopeptide of collagen I (ICTP), amino-terminal propeptide of procollagen I (PINP) osteocalcin (OC), and bone alkaline phosphatase (BALP) using enzyme immunoassay method (ELISA). Bone mineral density (BMD) was measured with dual energy X-ray absorptiometry (DXA). The study groups were compared using the unpaired t-test. All the data were expressed as mean ± SD at significance level of $p<0.05$. This pilot study has shown that metoprolol administration significantly increased the concentration of OC to 144 % ($p=0.002$). PINP, ICTP and BALP did not change significantly. The animals receiving metoprolol showed no change in BMD relative to the control group. Our findings suggest that metoprolol at dose of 0.5 mg/100 g BW may have potentially beneficial effects on bone metabolism in adult male albino Wistar rats by increasing the concentration of OC.

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TOXICITY TESTS PERFORMED AT UNIVERSITY OF VETERINARY AND PHARMACEUTICAL SCIENCES BRNO

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Research activities of the Department of Veterinary Public Health and Toxicology include toxicity studies on fish and amphibians. The selection of compounds for testing is based on results of monitoring of the aquatic pollution with pesticides and pharmaceuticals performed by Czech Hydrometeorological Institute. Another relevant information is consumption of plant protection products in the Czech Republic (provided by State Phytosanitary Administration). Most tests are conducted according to OECD procedures – No. 203 (Fish, Acute Toxicity Test), 210 (Fish, Early-Life Stage Toxicity Test), 212 (Fish, Short-Term Toxicity Test on Embryo and Sac-Fry Stages) and 215 (Fish, Juvenile Growth Test). Other, long-term studies on fish do not follow these standardised methods, however, their validity conditions are the same (dissolved oxygen concentration, constant concentration of the substance tested, constant temperature etc.). Test organisms are various developmental stages of *Danio rerio*, *Poecilia reticulata*, and *Cyprinus carpio*. The attention is paid also to toxic effects of xenobiotics to amphibians. The assessment is made using Frog Embryo Teratogenesis Assay – Xenopus on embryos of *Xenopus laevis*. Results of the studies contribute to the knowledge of adverse effects of xenobiotics on non-target aquatic organisms.

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SUBCHRONIC TOXICITY ASSESSMENT OF FUNGICIDE SPARTAKUS (PROCHLORAZ) USING COMMON CARP *CYPRINUS CARPIO*

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Toxic effects of a commercial pesticide formulation on a non-target organism were investigated in the study through biochemical, haematological, antioxidant and biometric indices, induction of xenobiotic metabolizing enzymes and histological examination of selected tissues. Common carp *Cyprinus carpio* were exposed to Spartakus (prochloraz 450 g/l) for 28 days, concentrations of prochloraz were 0.05; 0.15 and 0.38 mg/1. An increase in plasma potassium ($p<0.01$) was found in all test concentrations, there was a decline in total protein ($p<0.05$), Na^+, Ca and ALT ($p<0.01$) in fish treated with prochloraz of 0.38 mg/l. Ferric reducing ability of plasma decreased ($p<0.05$) in this concentration, while plasma ceruloplasmin activity was enhanced ($p<0.05$). A decrease in red blood cell count ($p<0.05$) was observed in prochloraz of 0.05 and 0.15 mg/1. There was a rise in hepatosomatic index ($p<0.01$), liver content of cytochrome P450 and activity of ethoxyresorufin-O-deethylase in fish exposed to prochloraz of 0.15 and 0.38 mg/l. Liver glutathione was found to increase in fish from prochloraz of 0.38 mg/l whereas activity of liver glutathione-S-transferase was induced by all concentrations. Treatment with all concentrations resulted in histological changes in gills, a decreased activity of skin mucous cells was detected in fish from prochloraz of 0.38 mg/l.

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PREDICTION OF EYE IRRITATION HAZARD USING IN VITRO ALTERNATIVE METHODS

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A special attention should be given to possible improvements through the evaluation of all existing information on the test substance in order to avoid unnecessary testing in laboratory animals. However to date, no single stand-alone *in vitro* method is validated to fully replace the conventional Draize eye irritation test. Since the EU ban on animal testing of cosmetic ingredients came into force in 2009, no animal experiments on eye irritation are authorized at all in this field. A key difficulty in determining the validity of alternative *in vitro* methods is that the *in vivo* animal data are both scarce, highly variable and often of limited utility for hazard prediction for man. We have analyzed available human and animal eye/skin irritation data with results of selected *in vitro* methods, including EpiOcular™, HET-CAM, Neutral Red Release or Uptake assays. Each of the *in vitro* methods was found to be related to a specific endpoint of ocular irritation and provided only partial information on the mode of action of the tested material. Despite good reproducibility of individual *in vitro* assays, only the weight-of-evidence approach and results of multiple selected *in vitro* tests can allow for estimation of the ocular effects *in vivo*. Each of the *in vitro* methods available so far provides only partial information related to the individual ocular tissue structures such as cornea, conjunctiva and/or

iris. None of *in vitro* alternatives can reproduce all the aspects of the *in vivo* method and thus are most likely to be used in combination or test batteries. To date, no single stand-alone *in vitro* method is validated to fully replace the conventional Draize eye irritation test. However, a key difficulty in determining the validity of alternative *in vitro* methods is that the *in vivo* animal data are both scarce, highly variable and often of limited utility for hazard prediction for man. In our presentation we have analyzed available human and animal eye/skin irritation data with results of selected *in vitro* methods, including HET-CAM, Neutral Red Release Assay, Neutral Red Uptake Assay and EpiOcular™ eye irritation test. Each of the *in vitro* methods was found to be related to a specific endpoint of ocular irritation and provides only partial information on the mode of action of the tested material. Despite good reproducibility of individual *in vitro* assays, only the weight-of-evidence approach and results of multiple selected *in vitro* tests can allow for estimation of the ocular effects *in vivo*. A special attention should be given to possible improvements through the evaluation of all existing information on the test substance in order to avoid unnecessary testing in laboratory animals. Where insufficient data are available, it is recommended that they be developed through application of sequential testing. The testing strategy includes the performance of validated and accepted *in vitro* tests. This method includes the recommendation that prior to undertaking the described *in vivo* test for acute eye irritation/corrosion, a weight-of-the-evidence analysis be performed on the existing relevant data. *In vivo* testing should not be considered until all available data relevant to the potential eye corrosivity/irritation of the substance has been evaluated in a weight-of-the-evidence analysis. In our presentation we have analyzed available human and animal eye/skin irritation data with results of selected *in vitro* methods, including HET-CAM, Neutral Red Release Assay, Neutral Red Uptake Assay and EpiOcular™ eye irritation test. Each of the *in vitro* methods was found to be related to a specific endpoint of ocular irritation and provides only partial information on the mode of action of the tested material. Despite good reproducibility of individual *in vitro* assays, only the weight-of-evidence approach and results of multiple selected *in vitro* tests can allow for estimation of the ocular effects *in vivo*.

LOCAL TOXICITY AND EMBRYOTOXICITY OF ORGANOPHOSPHATE INSECTICIDE (MALATHION) IDENTIFIED BY ALTERNATIVE IN VITRO METHODS

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Organophosphate insecticide Malathion is reported to be of low toxicity for man. Based on LD_{50} over 2000 mg/kg for rat and mice it is generally not classified as toxic. The toxicity seems to be species-dependent, particularly dependent on carboxylesterase activity that breaks down toxic malaoxon generated in the liver by cytochrome P450. Distinct data suggest also inhibition of the thyroid gland hormones, degeneration of ovary follicle cells and increase in the incidence of fetus resorption. Local and systemic toxicity data for birds are rare and/or ambiguous, but a decrease of wild bird densities in areas where malathion was applied has been repeatedly reported. The actual intoxication of wild birds may be influenced by the level of exposure from multiple sources, by age and sex of animals, by their state of nutrition and body condition. With the aim to extend knowledge on malathion toxicity on cellular and organ level we performed a number of experiments using progressive alternative *in vitro* methods that model local and systemic toxicity. The cytotoxicity was assessed in 3T3 fibroblast culture according to the ISO 10 993-5 standard. Skin and eye irritation potential was determined using reconstructed skin and eye cornea tissues (EpiDerm™ and EpiOcular™). As no skin and eye cornea irritation potential was demonstrated, the HET-CAM test using the rich vascular system of chorioallantoic membrane of chicken embryos in hen´s fertilized egg was utilized to detect effects on mucosa. The model of chick embryos was employed further for an extended study on acute embryotoxicity (mortality and genotoxicity) dependent on the time and place of malathion intra-embryonal application up to the day 8 of the chick embryo development. If malathion was applied to the amnion cavity, the chick embryo mortality was identified in lower doses, in higher incidence and in earlier stages of development in comparison with application to the air cavity. No genotoxicity was identified by means of micronucleus test in erythroid MNE I and MNE II cells isolated from chick embryo chorioallantois vascular system using morphological evaluation by optical and fluorescent microscopy. No significant changes in micronucleus and mitosis numbers were detected. The IC_{50} = 54.2±3.1 μg/ml obtained by the cytotoxicity *in vitro* test, which was recently validated as suitable to identify non-toxic, i.e. not classified substances, suggests higher toxic potential of malathion than is generally declared in literature based on conventional *in vivo* tests on laboratory rodents.

CHANGES IN LINKER BETWEEN PYRIDINIUM RINGS IN ACETYLCHOLINESTERASE REACTIVATORS PLAY THE IMPORTANT ROLE IN DISTRIBUTION AFTER IN VIVO APPLICATION

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The pharmacokinetic studies of AChE reactivators started by Wilson and Ginsburg investigations. They found that oximes are able to reactivate of alkylphosphate inhibited AChE. This basic information was soon outlined by recognizing of oximes absorption, distribution and elimination. K-series reactivators recently developed by Kuca and Musilek are the most promising groups of newly synthesized oximes at the present time. Among the newly synthesized reactivators, there are many structural homologues. The linker between two pyridinium rings may play role in reactivation potency and also in pharmacokinetics of AChE reactivators. A simple and reliable HPLC method for the determination of plasma levels of new acetylcholinesterase reactivators (K282, K075, K108 and K127) is widely presented in our study. The pharmacokinetic was evaluated in a Wistar rat model. Acetylcholinesterase reactivators separation was carried out by high performance liquid chromatography (HPLC) using octyl silica stationary phase and mobile phase consisting of 24 % acetonitrile, containing octane sulfonate sodium salt and tetramethylammonium chloride (pH 2.7-2.3). The calibration curves were linear in the range 1 – 100 μg/ml, which is well overlaying the detected plasma level ranges of therapeutical doses of acetylcholinesterase reactivators. After administration of equimolar doses of previously mentioned oximes, the significant differences in distribution were found. The linker between pyridinium rings in molecule of AChE reactivators is indisputably important factor that may influence the distribution of oximes after application and thus influence the efficacy of these therapeutics.

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SKIN AND EYE COMPATIBILITY OF COSMETIC PRESERVATIVES TESTED IN VITRO AND IN VIVO

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The study was focused on assessment of skin and eye irritation hazard of selected commercially available preservatives frequently used in cosmetic formulations. The classification of skin/eye irritation of concentrated chemical mixtures, specified by the manufacturers in the safety data sheets and based on rabbit Draize test results, was confirmed using *in vitro* methods (specifically skin irritation on reconstructed human skin model EpiDerm™, eye irritation on reconstructed human corneal model EpiOcular™ and on hen´s egg choriollantoic membrane HET-CAM). The hazard classification of the tested preservatives by means of *in vitro* methods was in total agreement with historical classification based on conventional test methods using experimental animals. The health safety of the selected preservatives in the highest concentrations recommended by the manufacturers was evaluated using the above mentioned *in vitro* methods and in case of skin irritation also

in a group of human volunteers (4h Human Patch Test). The *in vitro* methods confirmed the absence of eye irritation potential of all diluted preservatives with the exception of one mixture (containing phenoxyethanol 71.6 %, methylparaben 16 %, ethylparaben 4 %, propylparaben 2 %, butylparaben 4 % and isobutylparaben 2 %), which elicited a postive result in the eye irritation test on corneal model EpiOcular™. This result suggests, that an accidental massive exposure to a product containing the highest recommended concentration of this preservative may lead to eye irritation and/or corneal damage. The absence of skin irritation potential of all the tested preservatives in the highest recommended concentrations was confirmed by the battery of *in vitro* tests and consequently *in vivo* in a group of volunteers, verifying the safety of the recommended level of preservatives in the final formulations. In conclusion, the alternative *in vitro* methods based on organotypic models (HET-CAM) and reconstructed human tissue models seem to be a useful tool for the prediction of human eye and skin irritation, particularly for consideration of initial concentrations for confirmatory human patch tests to prove substance and product safety.

The study was supported by grant project of the Ministry of Health (No. NS9648-4/2008).

AETIOLOGIC AGENTS RECENTLY INVOLVED INTO HEALTH MONITORING OF LABORATORY RODENTS

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The last recommendation for the health monitoring of rodent and rabbit colonies in breeding and experimental units was issued by FELASA board June 9, 2001. Since that time a lot of work concerning to the infection diseases at laboratory rodents was done and published. Some of the most important agents are emphasized at virology: *Rat parvovirus (RPV1), Rat minute virus (RPV2), Murine norovirus, Rat rota virus (IDIR)*, at bacteriology *Pasteurellaceae* and at mycology *Pneumocystis sp.* A development of molecular biological methods significantly contributed to precising of infection agents. On the other hand it made more complicated routine dignostic. This is evident in the case of *Pasteurellaceae*- family of Gram negative bacteria with members from comensal microflora to pathogenic germs – where exists more than 15 genera of *Pasteurellaceae*. According to FELASA recommendation all *Pasteurellaceae* should be monitored and mentioned in the Health report. AnLab has examined of 1452 mice and rats in the year 2010. The results of *Pasteurellacae* detection in trachea (T), colon (C) and vagina/prepuce (V) were following: *Pasteurella pneumotropica* T30, C11, V143; *Actinobacillus muris* T9, C4, V49; *Haemophilus influenzae* T1, C0, V5. No any other *Pasteurellaceae* genera were detected. It is evident a pathogenecity of newly classified germs should be determined. *Pneumocystis sp.* was found many years ago and defined as *Protozoa* later was transferred to Fungi *(Ascomycota)*. The surprising discovery its responsibility for infectious interstitial pneumonia at rats previously attributed to *Rat respiratory virus* was done in the last year. Methods of detection and pathological changes of lungs are described.

DEMONSTRATION OF THE PRESENCE AND FREQUENCY OF *HELICOBACTER SP.* DETECTED IN LABORATORY MICE

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A polymerase chain reaction (PCR) belongs to preferred method for detection of *Helicobacter sp*. in comparison with histopathology, serology and cultivation. The importance of regular *Helicobacter sp.* PCR detection in laboratory mice is supported by our laboratory results between the years 2009-2010. In the year 2009, 277 mice from Czech facilities and 658 mice from European facilities were examined with 66 positive results in Czech Republic and 139 positive results in other facilities in Europe. Next PCR differentiation confirmed presence of pathogenic *Helicobacter hepaticus*. There were 56 % in Czech Republic and 51 % in European facilities. In the year 2010, 244 mice from Czech facilities were examined with 59 positive results 1102 mice from European facilities with 311 positive results of *Helicobacter sp*. *Helicobacter hepaticus* was confirmed at 52 % of animals (Czech facilities) and 37 % (European facilities) respectively. From our results the wide spreading of *Helicobacter* is evident. We have to emphasize the fact that part of samples originate from experimental facilities with barrier systems. The results are supported by our short study at different population of wild mice in the year 2008, where majority of tested animals were positive for *Helicobacter hepaticus*.

USE OF LABORATORY ANIMALS IN PRECLINICAL RESEARCH ON DRUG ADDICTION

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Animal models for experimental study of drug addiction are intended for testing of the whole range of drugs of abuse (e.g. opioids, amphetamines, ecstasy, cocaine or tetrahydrocannabinol). Animal models of addiction are created according to characteristic symptoms in human addicts and enable observation of behavioural changes and also neurobiological analyses. The most widespread behavioural methods can be divided into models of the positive reinforcing drug effects and into models of aversive effects of drug withdrawal. Models of the positive reinforcing effects of drugs include: "Operant drug self-administration" (particularly intravenous), reward caused by stimulation of the brain reward pathway (Ventral Tegmental Area – Nucleus Accumbens – Prefrontal Cortex), "Place preference" and drug discrimination. Conditioned place aversion can be named as a typical model of aversive effects of drug withdrawal. Other useful tools for testing of phenomena associated with drug abuse potential are represented by "Open field test" or model of agonistic behaviour in rodents. These two models were proven in our behavioural laboratory, especially in experiments focused on phenomenon of behavioural sensitization to psychostimulant methamphetamine. Behavioural sensitization is conditioned by repeated administration of various drugs of abuse, leads to an increased behavioural response and is considered as a possible cause of relapses in ex addicts. Thus, particularly the possibility to measure methamphetamine stimulatory effects on locomotor/exploratory behaviour in the "Open field test" has been shown very beneficial for the above mentioned purposes. In generally, results obtained from the animal models of addiction fulfil criteria of translational research of drug addiction problems in humans. Moreover, application of these models can serve for estimation of addictive potential of the drug tested as well as prediction of possible pharmacological treatment of its abuse.

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SPONTANEOUS ABDOMINAL HEMANGIOSARCOMA OF A LEWIS RAT

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Laboratory rodents are one of the most common experimental models used in biomedical research. The quality of laboratory animals has a major influence on reproducible animal experiments. Laboratory animals should be therefore standardized as much as possible. Spontaneous neoplasia (benign and malignant) is one of the most reasons for upset health conditions of laboratory animals and eventually can lead to their death. In this study we report a case of spontaneous abdominal hemangiosarcoma in female Lewis rat.

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THE EFFECT OF PINWORM INFECTION WITH *SYPHACIA MURIS* (YAMAGUTI, 1935) ON LABORATORY RATS' BODY WEIGHT GAIN

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Laboratory rodents are one of the most common laboratory animals used in biomedical research. Therefore both the quality and reliability of laboratory animals have a major influence on research. That is the reason why animals that are standardized as much as possible are important prerequisites for reproducible animal experiments. Only healthy, well provided for animals yield valid scientific data and thus oblige us to the highest standards of care. Pinworms (Nematoda: Oxyurida) are common contaminants in most laboratory rodent colonies. Every conventional colony is probably infected with oxyurids. Sometimes pinworm infections can occur in SPF (specific pathogen-free) rodent colonies, too. Although pinworms of laboratory rodents are generally considered relatively non-pathogenic, and infections are generally regarded as symptomless, non-specific clinical signs appear even in heavy infections. This study show how can pinworm infection influence the body weight gain in rats naturally infected with *Syphacia muris* in compares with laboratory rats which are pinworm infection free.

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SOLAR WATER DISINFECTION VERSUS *LEPTOSPIRA SP.*

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Solar water disinfection, also known as SODIS is a method of disinfecting water using only sunlight and plastic PET bottles. SODIS is a free and effective method for decentralized water treatment, usually applied at the household level and is recommended by the World Health Organization as a viable method for household water treatment and safe storage. UV-A (wavelength 320-400 nm) reacts with oxygen dissolved in the water and produces highly reactive forms of oxygen (oxygen free radicals and hydrogen peroxides), that are believed to damage pathogens (from Wikipedia). UV-A can cause mutation in bacterial DNA, and thermal effect of infra red radiation is necessary to mention. Our experimental work has been engaged with SODIS vs. well water contaminated by leptospira strains (density 300 cells per ml; *Leptospira grippotyphosa* P125, *Leptospira Copenhageni* Lebe). This technique has not ever been published in scientific literature. After repeated exposition (48 hours) of water samples (15 bottles) to solar radiation, 100 ml of water was sampled, cetrifuged and consequently tested by PCR. All samples were positive. Viability of leptospira was proven on laboratory animal mice model, when we didn't have good results in microscopy (some ballast and contaminants) after 21 days cultivation. Mice (28 female BALB/c, 6-8 weeks old) were infected with suspension of leptospira in liquid Korthof's medium and were exposed to agents by gastrointestinal administration. Positive control: $2x10^6$/ml leptospira in 500 µl Korthof's medium. After death of mice (28 days) we froze spleen, liver, urocyst and kidney for PCR testing.

USTÁJENÍ LABORATORNÍCH ZVÍŘAT V IVC STOJANECH

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V rámci řešení projektu bezpečnostního výzkumu „Cílený vývoj léčiv použitelných k ochraně obyvatelstva před bioterorismem. Vývoj a studium účinných inhibitorů adenylátcyklasového toxinu patogenů *Bordetella pertusis* a *Bacillus anthracis*", jehož poskytovatelem je Ministerstvo vnitra, byl v závěru r. 2011 pořízen systém stojanů a ventilační jednotky pro ustájení myší a potkanů – individuálně ventilované boxy (IVC), včetně příslušenství. Zařízení se skládá z ventilační jednotky, stojanu pro umístění klecí GR900 pro potkany a myši o ploše 900 cm^2 (celkem 35 boxů), stojanu pro umístění klecí GR500 pro myši o ploše 501 cm^2 (celkem 70 boxů), ochranného boxu pro manipulaci se zvířaty a boxu pro manipulaci a likvidaci použité podestýlky. Dodavatelem je firma TRIGON PLUS, s.r.o., výrobcem firma TECNIPLAST, S.p.a., Italy. Prezentace uvádí základní technické informace o zařízení a prvotní poznatky a zkušenosti z jeho reálného provozu.

TOXICITY ASSESSMENT OF TERBUTRYN USING FISH TESTS

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This study documented differences in sensitivity of several fish species and different developmental stages of fish to terbutryn. Terbutryn belongs to symmetrical triazine herbicides used extensively in agriculture and non-agricultural sites (primarily to control broadleaf and some grassy weeds) that have become ubiquitous contaminants of the environment. Tests of terbutryn toxicity were performed on the aquarium fish *Danio rerio* and *Poecilia reticulata*, which are the model organisms most commonly used in toxicity tests. The acute toxicity tests on juvenile stage of *D. rerio* and *P. reticulata* were performed according to the method OECD No. 203 Fish, Acute Toxicity Test. Toxicity tests on the embryonic stage of *D. rerio* were performed according to the method OECD No. 212 Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages. The study proved significantly higher ($p<0.01$) sensitivity of the juvenile stage of *D. rerio* to terbutryn compared to the embryonic stage of *D. rerio* and significantly higher ($p<0.01$) sensitivity of the juvenile stage of *P. reticulata* to terbutryn compared to the juvenile stage of *D. rerio.*

TOXICOLOGICAL SCORING OF ALZHEIMER´S DISEASE DRUG HUPERZINE IN A GUINEA PIG MODEL

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Huperzine is a secondary metabolite in lycopods *Huperzia* and an inhibitor of acetylcholinesterase. Besides acting as inhibitor, it is also an antagonist of N-methyl-D-aspartate receptor. Huperzine is a suitable drug for the treatment of Alzheimer´s disease as it is a part of traditional Chinese medicine. Currently, it undergoes clinical trials in the European Union and United States. The toxicological data about huperzine are missing and link between huperzine and oxidative stress has not been extensively investigated. For the above mentioned reasons, we organized experiment on a guinea pig model aimed at the investigation of adverse effects caused by huperzine. Guinea pigs were exposed to (-)-huperzine A in doses 5-625 µg/kg. Animals were sacrificed one day after exposure. Ferric reducing antioxidant power, thiobarbituric acid reactive substances, glutathione reductase, caspase 3 activity and

selected biochemical markers (e.g. transaminases, blood urea nitrogen and glucose) were assayed. In frontal, parietal, temporal lobes and cerebellum, we found increase of antioxidants, glutathione reductase and oxidative stress markers in a dose dependent manner. Effects on liver, kidney and spleen were milder. We discuss ambivalent action of huperzine in the body and judge the huperzine action owing to recently reported experiments.

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GENETIC CONTROL OF RESISTANCE TO *TRYPANOSOMA BRUCEI BRUCEI* INFECTION IN MICE

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Trypanosoma brucei brucei infects livestock, with severe effects in horses and dogs. Mouse strains differ greatly in susceptibility to this parasite. However, no genes controlling these differences were mapped. To study genetic control of survival after *T. b. brucei* infection we used recombinant congenic (RC) strains. Each RC strain of BALB/c-c-STS/A (CcS/Dem) series contains a different random subset of 12.5 % genes from the parental "donor" strain STS/A and 87.5 % genes from the "background" strain BALB/c. Although BALB/c and STS/A mice are similarly susceptible to *T. b. brucei*, the RC strain CcS-11 is more susceptible than either of them. We analyzed genetic influence on survival in *T. b. brucei*-infected F_2 hybrids between BALB/c and CcS-11. CcS-11 strain carries STS-derived segments on eight chromosomes. We genotyped these segments in the F_2 hybrid mice and tested their linkage to survival by analysis of variance. Using this method we mapped four new loci which influence survival after *T. b. brucei* infection. We call them *Tbbr* (*Trypanosoma brucei brucei* response) loci. *Tbbr1* (chromosome 3) and *Tbbr2* (chromosome 12) have effects on survival independent of inter-genic interactions (main effects). *Tbbr3* (chromosome 7) influences survival in interaction with *Tbbr4* (chromosome 19). This study presents the first identification of chromosomal loci controlling susceptibility to *T. b. brucei* infection. While mapping in F_2 hybrids of inbred strains usually has a precision of 40-80 Mb, in RC strains we mapped *Tbbr2* to a 2.15 Mb segment containing only 26 genes, which will enable an effective search for the candidate gene. Definition of susceptibility genes will improve the understanding of pathways and genetic diversity underlying the disease and may result in new strategies to overcome the active subversion of the immune system by *T. b. brucei*.

Šíma M, Havelková H, Quan L, Svobodová M, Jarošíková T, Vojtíšková J, Stassen APM, Demant P, Lipoldová M: Genetic control of resistance to Trypanosoma brucei brucei infection in mice. *PLoS Neglected Tropical Diseases*, 5, e1173, 2011.

BONE METABOLISM, BLOOD WITHDRAWALS AND IRON ENRICHED DIET IN RATS

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Voluntary blood donors are usually determined to by healthy without any serious illness. Blood donors are usually given iron supplementation to compensate for iron loss resulting from whole-blood donation. However there are no many studies about the effect of repeated blood withdrawals on bone and their metabolism. The aim of our study was to evaluate the effect of repeated blood withdrawals with different iron enriched diets on bone metabolism in rats. The healthy male Wistar rats (n=48) at the age of 8 weeks were divided into 6 groups with 8 animals in each. The animals were fed either standard laboratory diet (SLD) or SLD supplemented with iron, and that was either 400 mg elementary iron/1 kg diet (fe) or 5 g elementary iron/1 kg diet (FE+). Half of animals had blood withdrawals (w) every week, totally 8times. Half of millilitre of blood per 100 grams body weight were taken from retro-orbital sinus. Groups of animals: 1. SLD, 2. SLD-w, 3. fe, 4. fe-w, 5. FE+, 6. FE+w. In serum were evaluated concentration of markers of bone formation: osteocalcin (OC), amino-terminal propeptide of procollagen I (PINP) and markers of bone resorption: carboxy-terminal teloptide of collagen I (ICTP) using an enzyme immunoassay method (ELISA). Bone mineral density (BMD) was measured with dual energy X-ray absorptiometry in three parts of rat`s body: femur, lumbar and tail vertebrae. The significance between the groups with and without repated blood withdrawals was analysed by the unpaired t-test. The data were presented as means and standard deviation (SD). Concentration of bone markers, PINP, OC and ICTP, increased in all groups with blood withdrawals compared to the groups without blood withdrawals (SLD-w x SLD, fe-w x fe, FE+w x FE+), but there were no statistically significance. BMD was decreased only in FE+w vs. FE+ in femur, lumbar and tail, but not significantly. Even though there were no significant differences, our results indicated increase in bone turnover due to repeated blood withdrawals in all groups-SLD-w, fe-w and FE+w. This may be related to stimulation of haematopoiesis in bone marrow, which is probably the effect of repeated blood withdrawals. The unanswered question remains why bone mineral density decreased in all parts only in FE+w group.

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EFFECT OF AMINO ACIDS ENRICHED DIET ON BONE METABOLISM IN RATS

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Branched-chain amino acids (BCAA) are essential acids that make up about 1/3 of skeletal muscles in human body and play important role in the synthesis of body proteins. Glutamine is non-essential amino acid, which is part of proteins and play role in ammonium detoxication. The aim of this study was to investigate the effect of selected amino acids added to diet on bone metabolism in rats. Male Wistar rats were divided into 4 groups of 6 animals and fed for three months by a different types of diets. 1. group (CO) were controls fed standard laboratory diet (SLD), 2. group (CAS) were fed casein-enriched diet (100 g SLD + 19.7 g kasein), 3. group (BCAA) were fed branched-chain-enriched diet (100 g SLD + 28.7 g BCAA compound of: 50 % leucine, 25 % valine, 25 % isoleucine), 4. group (GLN) were fed glutamine-enriched diet (100 g SLD + 16.6 g glutamine). Animals were weighted every second day. We evaluated marker of bone resorption: carboxy-terminal telopeptide of collagen I (ICTP) and markers of bone formation: osteocalcin (OC) and amino-terminal propeptide of procollagen I (PINP) by an enzyme immunoassay method (ELISA). Bone mineral density (BMD) was measured by dual energy X-ray absorptiometry. Statistical analysis was performed by one-way ANOVA using SigmaStat software (Jandel Scientific, USA). Results are presented as means followed by standard deviation (SD). Differences of $p<0.05$ were considered significant. BCAA group increased body weights ($p<0.05$) only to 67 % of control´s value. BMD in tail part ($p<0.05$) decreased to 86 % as compared to CO. Concentration of ICTP ($p<0.05$) decreased to 69 % in BCAA group and to 67 % in GLN group as compared to CO. BCAA led to not significant decrease of all markers of bone metabolism and BMD in all measured parts as compared to CO. There are no differences between CAS and CO groups. The poor increase in body weights in BCAA group was probably evocated by unattractive branched-chain-enriched diet. Bone turnover was lowered in both, BCAA and GLN group, but it led to decreased bone mineral density only in BCAA group. We suppose, that this discrepancy in BMD was due to weight loss, not due to amino-acids enriched diet. Other studies with pair-fed animals are necessary to investigate the effect of BCAA on bone metabolism in rats.

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SILYMARIN AFFECTED EXPRESSION OF ABC TRANSPORTERS IN THE RAT

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Silymarin (standardized extract from the seeds of the *Silybum marianum*) has been used in supportive therapy of liver diseases and its cytoprotective activity is believed to be based on antioxidant properties. Our previous works showed hypolipidemic effects of silymarin. The ATP-binding cassette (ABC) transporters G5 and G8 play a main role in biliary cholesterol secretion. The ABCA1 transporter plays a significant role in movement of cellular cholesterol to high density lipoproteins. The possibility that silymarin affects the regulation of lipid metabolism *via* selected ABC transporters has been studied. The rats were fed (*ad libitum*) for 20 days on a standard laboratory diet (KrmiMo Mohelsky, Czech Republic) or on an experimental high-cholesterol diet prepared by adding 1 % (w/w) of cholesterol to the standard laboratory diet. Silymarin (1 % or 3 %, w/w; Sigma Aldrich) was administered as a dietary supplement to the high-cholesterol diet. All experiments with animals were approved by the Ethics Committee from the Ministry of Education, Czech Republic. The major finding in this study is that silymarin up-regulated mRNA of ABC transporters (G5, G8 and A1). This result suggested that silymarin positively affects plasma lipoprotein profile *via* up-regulation of ABC transporters connected in lipid metabolism. Furthermore, this study shows for the first time that silymarin up-regulated expression of this ABC transporter's mRNA.

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EXPERIENCE IN IDENTIFICATION OF EXPERIMENTAL AMPHIBIANS AT THE UNIVERSITY OF VETERINARY AND PHARMACEUTICAL SCIENCES BRNO

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Aquatic species of amphibians are notoriously difficult to mark or individually identify. Their usually small physical size, sensitive and slippery skin, necessary subtlety of their markings, and their ability to regenerate toes all contribute to these difficulties. Among worldwide the most often used methods for amphibian permanent marking rank hot, freeze or chemical branding, tattooing, natural marking identification, tissue removal, passive integrated transponders and visible implant fluorescent elastomer tags. In the Czech Republic some of these methods are banned according Act No. 246/1992 Coll. on the protection of animals against cruelty, as amended. A group subadult and adult African clawed frogs (*Xenopus laevis*) have been marked and identified by different permanent methods. In these frogs, schemes for identification of each individual were needed to follow their artificial hormonal breeding stimulation and use of their eggs and embryos in ecotoxicologic biotests. At the beginning with non-invasive natural marking identification was started. First photographs of each individual were taken under standard conditions. Features used for identification included sex and size of each frog, and colour, size, shape, location and configuration of spots on the skin. This method proved to be suitable for individuals with natural colour with a large number of spots, while completely useless in albiniotic frogs. Passive Integrated Transponders (PITs) are small electronic units encased in biologically inert glass capsules with a diameter of 2 mm and length about 20 mm. In twenty frogs PIT tags were implanted under general anaesthesia (ketamine at the dose of 90 mg/kg). An incision was made in the middle of the back and the transponder was injected into the dorsal lymph sac. The wound was closed with one silon suture or with surgical glue. Five of the twenty transponders were not readable after two months, as they were lost through the puncture hole after injection (two) or were rejected because of small size of the individual (three). Recently, we have begun to experiment with a promising new technique using visible implant fluorescent elastomer tags (VIE). VIE tags implanted in the translucent skin between the toes seems to be very successful for long-term individual marking. The advantages of the system include the large number of individuals that could be marked, high mark retention, mark visibility under ambient or UV light and the low volume of the tags.

ROSUVASTATIN AFFECTED CYTOCHROME P450 2C11 IN HEALTHY RATS

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HMG-CoA reductase inhibitors (statins) are important drugs in the therapy of dyslipidemia. Since, they are widely prescribed, their safety remains of great interest. Several available statins are metabolized by cytochrome P450 (CYP) 3A4 and can therefore interact with commonly used medications. Rosuvastatin like synthetic hydrophilic statin undergoes only minor metabolism by cytochrome P450. The aim of our study was investigate the effects of rosuvastatin on liver expression of cytochrome P450 isoform 2C11 in rat. Male Wistar rats were fed for 3 weeks (*ad libitum*) on a standard laboratory diet (STD), on an experimental high-cholesterol diet (HCD) prepared by adding 1 % (w/w) of cholesterol and 10 % (w/w) of lard fat to the STD. Rosuvastatin (RS 0.01 %, w/w and RS 0.03 %, w/w) was administered as a dietary supplement to the HCD. Rats were then fasted overnight, i. m. anesthetized and their liver was removed and frozen. Expression of CYP2C11 mRNA was measured by Real-Time PCR. The following primer sequences were used: CYP2C11 Fw 5´-TGA GGA AGA GCA AAG GTG CCC CT-3´ and CYP2C11 Rev 5´-ATT GCA GAC CTG TAG CCA TGG GGA-3´. Our results demonstrate a significant down regulation of hepatic microsomal CYP2C11 mRNA after feeding of both concentrations (0.01 % and 0.03 %) of rosuvastatin. We suggested that reduction of rat CYP2C11 (consequently human CYP2C9) expression by rosuvastatin may be important for drug-drug interactions of some drugs such as diclofenac or warfarin.

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FENOFIBRATE AFFECTED CYTOCHROME P450 2C IN HEALTHY AND HEREDITARY HYPERTRIGLYCERIDEMIC RATS

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Influence of fenofibrate (used in therapy of hypertriglyceridemia) on expression of liver mRNA of cytochromes P450 (CYP2C6 and CYP2C11) in hereditary hypertriglyceridemic rats (HHTg, accepted model of metabolic syndrome) and healthy Wistar-Kyoto (WKY) rats was studied. The rats were fed for 20 days (*ad libitum*) on a standard laboratory diet (KrmiMo Mohelsky, Czech Republic) or on an experimental high-cholesterol diet prepared by adding 1 % (w/w) of cholesterol to the standard laboratory diet. Fenofibrate (0.1 %, w/w; Fournier, France) was administered as a dietary supplement to the high-cholesterol diet. Real time PCR confirmed that CYP2C6 and CYP2C11 mRNA in liver was down-regulated by fenofibrate in healthy and HHTg rats. Conclusions: this work shows that (i) fenofibrate affect expression of CYP2C6 (this cytochrome P450 is similar to human CYP2C19 which plays a key role in metabolism of drugs, e.g. diazepam or propranolol) and (ii) fenofibrate also affects expression of CYP2C11 (similar to human CYP2C9 which plays important role in metabolism of some widely used drugs, e.g. diclofenac or S-warfarin). Considering that fenofibrate is a widely used hypolipidemic drug our results suggest that down-regulation of P450 2C mRNA may be important in drug-drug interactions. All experiments with animals were approved by the Ethics Committee of the Ministry of Education, Czech Republic.

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HPLC STUDY OF BLOOD-BRAIN BARRIER PENETRATION OF TWO ACETYLCHOLINESTERASE INHIBITORS – TACRINE AND 7-METOXYTACRINE (7-MEOTA) IN RATS

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The Alzheimer disease is a slowly progressive neuropsychiatric illness of unknown etiology. This illness is characterized by a progressive loss of cognitive ability and other intellectual functions. The most surveyed neurochemical imbalance is a deficit of enzyme choline acetyltransferase (EC 2.3.1.6) in brain, enzyme malfunction leads to impairment of acetylcholine synthesis, an important neurotransmitter. The lower level of acetylcholine in synapses may be increased by inhibition of enzyme acetylcholinesterase (AChE; EC 3.1.1.7). The AChE inhibitors remain the key drugs in the treatment of Alzheimer disease. In the present study, basic information about two AChE inhibitors, tacrine and its derivate 7-MEOTA (7-methoxytacrine) was characterized. 7-MEOTA is a potent, centrally active cholinesterase inhibitor with severalfold lower acute toxicity when compared to tacrine. 7-MEOTA also does not influence cognitive functions of treated animals. Both tested AChE inhibitors were applied orally in equimolar doses. These doses correspond with therapeutic dose (5 % LD_{50}) of tacrine. 7-MEOTA was also applied in its therapeutic dose (37.65 mg/kg). Following oral administration of tacrine equimolar doses (5.15 mg/kg) and 7-MEOTA (5.01 mg/kg) the distribution thru the blood-brain barrier was different. Tacrine brain concentration was relatively high and corresponds with 13.1 % of plasma level (real concentration 7.52±2.41 ng/g), but the 7-MEOTA brain concentration after application of equimolar dose was significantly lower (3 %; 0.17±0.09 ng/g). Levels of both AChE inhibitors in the brain tissue were comparable only if the 7-MEOTA was applied in its therapeutic dose. The real brain concentration of 7-MEOTA applied in therapeutic dose was 2.25±0.46 ng/g that was 4.3 % of plasma level.

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IDIOPATHIC LIVER NECROSES IN LABORATORY MICE

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Focal or multifocal coagulation liver necroses occur relatively often as the incidental finding at health monitoring in laboratory mice from SPF facilities. No pathogens were found and no intervences to the homeostasis of organisms were detected to cause their development. Their spontaneous occurence appears to be age and strain independent. Our laboratory noticed cca 0.5 % incidence of liver necroses during the last 2 years in otherwise healthy mice coming from czech and abroad facilities. The characteristically solitary or multiple small, irregular, sharply demarcated, pale foci in the liver were histologically coagulation necroses with or without inflammatory infiltrate. Etiology of these alterations was further microbiologically, parasitologically and histopathologically investigated in the range overlapping the FELASA recommendations. Up to now diagnostic methods have failed to prove any cause resposible for development of liver necroses. *MHV* infection, *Tyzzer´s disease*, *Helicobacter spp.* infection, clostridial enterotoxemia, other known viral and bacterial infections, parasital invasions, causes leading to the ischemia, neoplastic processes and affection by chemicals were not confirmed differential-diagnostically. In the future, diagnostic efforts should be focused on other possible causes of liver necroses; among them ischemia, toxins, endotoxins, biliary obstructions, microbial activity and their pathogenesis and impact of barrier technologies should be considered.

MURINE NOROVIRUS – INTERFERENCE WITH RESEARCH AND PREVALENCE

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Murine norovirus is the most prevalent viral agent in laboratory mice facilities throughout the world. The importance of norovirus infection in mice lies in possible affection of immunological, gastrointestinal studies and other trials, in which evaluation is based on histological examination. None of the available reports deals with the situation in the Czech Republic. The data shown in this study are collected from examination of 327 laboratory mouse sera from 5 facilities in Czech Republic and 1943 laboratory mouse sera from 33 mostly European facilities. Sera were examined by enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFA) in the time period of 2 years; for 2009 and 2010. Based on the results obtained, the prevalence determined for Czech Republic was 8.6 % for 2009 and 5.7 % for 2010; and for abroad it was 26.6 % for 2009 and 21.7 % for 2010. The literature (2009) documents 24-32 % prevalence for European facilities. Resulting prevalence rates for the Czech Republic are very different from the prevalence rates found for the abroad, mostly European facilities. Suspect distorsion of results can be most likely caused by a low number of Czech facilities which investigate the presence of this infection in their mice periodically.

THE EFFECT OF MUD-BATHS ON BONE MINERAL DENSITY IN MALE RATS

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We studied the influence of bath-mud application on subchronic arthritis and bone mineral density on Wistar male rats after 50 days. The arthritis was induced by a subplantar injection (100 µl of suspension Freund´s adjuvans with heat-killed *Streptoccocus pyogenes B stock*) into the plantar surface of the right hind paw on 1st and 8th day of experiment. Intact animals were similarly injected with saline solution. The 30 rats were divided into 5 groups. They were 34 bath for 20 minutes 4-5times in week: on dry chippings (21 °C), on hot dry sand (38 °C), and on hot or mild vet mud (38 or 21 °C). 1. group: intact (INT) on dry chippings, 2. group: (CONT) with arthritis on dry chippings, 3. group: (SAND) with arthritis on hot dry sand, 4. group: (mud38) with arthritis on hot vet mud, 5. group: (mud21) with arthritis on mild vet mud. The rats were sacrificed exsanguination after 50 days. Then *post mortem* bone mineral density (BMD) was measured with dual energy X-ray absorptiometry (DXA) in all rats. The pads for histopathological examination were fixed in 10 % buffered formalin with formic acid. We analyzed blood cell count in fresh heparinized blood by Abbott CELL-DYN 3200 SL (Abbott, IL, USA). Circulating immune complexes (CIC, unit) were determined in serum. Statistical analyses were performed by software "SigmaStat 3.1" Jandel Scientific®, San Rafael, CA, USA. All the data were expressed as mean ± SEM ($p<0.05$). The subchronic arthritis led to lower concentration of hemoglobin and lower leukocyte count, and higher neutrophile count. The lower CIC (2 vs. 11 in CONT) and grade of arthritis were in group mud21. The higher BMD in all regions of interest were in group mud38 (spine/femur/tail: 0.227±0.008 / 0.175±0.006 / 0.220±0.004 vs. CONT 0.216±0.006 / 0.150±0.007 / 0.214±0.006). Our findings suggest that male rats with baths in hot vet mud had higher BMD. Positive effect of mild tempered vet mud was on healing of arthritis and moderately on BMD vs. control group.

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ASSESSMENT OF BONE TISSUE IN LABORATORY ANIMALS

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The abstract deals with current opinions in assessment of bone tissue in laboratory animals. The bone resistance is estimated by densitometric analysis (DEXA – dual energy X-ray absorptiometry, or analyzes based on the principles of CT or MR – high-resolution micro-computed tomography, magnetic resonance imaging). Before measurements, a tissue calibration scan is performed with the phantom for the small animal. BMD of the whole body, in the lumbar and tail vertebrae and in diaphysis of femurs, and total lean and fat masses were evaluated by computer using the appropriate software program for small animals. To test the mechanical strength of the bones are used in commercially available instruments or "custom-made material testing machine". The most commonly used method is a method of three-point bending of bone. Tested bone is placed on two supports spaced at a certain distance, the third upper blade, applied perpendicular to the center shaft of the bone. The device records the force required to break the bone. Torsion test gives information about the torque. For this test, both bone ends are attached to the brackets and one turns to the broken bone. Compression test is used especially for vertebrae and femoral neck. The test can be performed on all types of bone and in different orientations. The bone markers producing by bone served for evaluation of bone metabolism. The osteocalcin (OC), procollagen type I N-terminal propeptide (PINP), C-terminal crosslinking telopeptide of type I collagen (CTX-I), alkaline phosphatase (ALP) and bone morphogenetic factors (BMFs) are released from the bone tissue locally. Bone segments are disrupted in homogenizer. The supernatant is used for the analysis of bone markers by commercial rat ELISA kit.

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THE EFFECT OF HOT SAND AND MUD-BATHS IN FEMALE RATS

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We studied the influence of 34 bath-mud applications on subchronic arthritis and bone mineral density in Wistar adult female rats. The arthritis was induced by subplantar injections (100 µl of suspension Freund´s adjuvans with heat-killed *Streptoccocus pyogenes B stock*) into the plantar surface of right hind paw on 1st and 8th day of experiment. Intact animals were injected by saline solution. The rats were divided into 5 groups (6 in each group) and were 34 baths, 20 minutes 4-5times in week: on dry chippings (21 °C), on hot dry sand (38 °C), and on hot/mild vet mud (38/21 °C). 1. group: intact (INT) on dry chippings, 2. group: (CONT) with arthritis on dry chippings, 3. group: (SAND) with arthritis on hot dry sand, 4. group: (MUD38) with arthritis on hot vet mud, 5. group: (MUD21) with arthritis on mild vet mud. The rats were sacrificed exsanguination after 50 days. Then *post mortem* bone mineral density (BMD) was measured with dual energy X-ray absorptiometry (DXA). The pads for histopathological examination were fixed in 10 % buffered formalin with formic acid. We analysed blood cell count in fresh heparinized blood by Abbott CELL-DYN 3200 SL (IL, USA). We analyzed serum circulating immune complexes (CIC, unit). Statistical analyses were performed by software "SigmaStat 3.1" Jandel Scientific®, San Rafael, CA, USA. The data were expressed as mean±SE ($p<0.05$). The subchronic arthritis decreased hemoglobin concentration (g/l; INT 152±2; CONT 147±4; SAND 145±2; MUD38 149±3; MUD21 144±3) and leukocyte count ($x0^9$/l; INT 10.5±0.7; CONT 6.4±0.9; SAND 6.7±1.1; MUD38 7.1±0.7; MUD21 7.3±0.4) but increased neutrophile count (%; INT 11.8±2.8; CONT 24.7±2.3; SAND 21.3±3.2; MUD38 21.0±2.5; MUD21 17.9±4.8). The lower CIC (0.5 vs. 14), better arthritis healing and spine BMD (0.205±0.008 vs. 0.203±0.002) were in group SAND vs. CONT. The lower BMD in spine and femur were in group MUD38 (0.196±0.004/0.119±0.007 vs. CONT 0.203±0.002/0.136±0.006; but no in tail (0.177±0.005 vs. 0.169±0.006). The group SAND had better healing of arthritis and lower decrease of BMD, mainly in spine. The decrease of BMD was in group MUD38. We suppose positive effect of mild tempered mud on healing of arthritis and BMD rather than hot mud.

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