of GEN protection of diabetes development in female NOD mice is complex, and more studies are needed to identify the immune components that are targeted by GEN (Supported in part by NO1-ES05454).

PS 1514 ACETAMINOPHEN REVEALS IMMUNE-SENSITIZATION IN ORAL EXPOSURE MOUSE MODEL.

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Many drugs are known to induce adverse immune reactions in susceptible individuals and may result in clinical diseases. The reporter antigen popliteal lymph node assay (RA-PLNA) is assessing this immune-sensitization capacity of low molecular weight compounds. However this assay only assesses local responses and does not include oral exposure. Therefore systemic effects are missed and furthermore, when metabolism is required compounds may test false negative in this assay. Preclinical systemic exposure models with a predictive value do not exist.
In this study the immune-sensitization of acetaminophen (APAP) was investigated when orally administered to mice. Trinitrophenyl-ovalbumin (TNP-OVA) was used as a reporter antigen (RA) to assess the capacity of the drugs to stimulate systemic immune responses to a bystander antigen. Female C3H/HeOuJ mice were exposed to 30, 100 or 300 mg/kg APAP daily for 7 days and co-exposed to TNP-OVA at day 1. 15 days after the start of the exposure delayed type hypersensitivity was determined by a challenge with the RA. A vigorous response to the RA was detected in animals exposed to APAP. RA-specific IgG1 and IgG2a antibodies could be detected in the serum at day 21. Furthermore, isolated LN cells from APAP treated animals displayed a higher IFN-γ, IL-4 and TNF-α production than cells from control animals upon restimulation in vitro. In conclusion, APAP, with a known capacity to induce immune mediated adverse reactions in patients, displays in this oral exposure mouse model a mixed TH1/Th2 response. Combined with results from other studies in which ofloxazine, diclofenac and D-penicillamine, all known immune-sensitizing drugs and induced antibody responses in this mouse model, this experimental set up could be useful for preclinical assessment of sensitizing potential of new chemical entities.

PS 1515 EXAMINATION OF POTENTIAL CHEMICAL INTERACTIONS IN SKIN SENSITIZATION : ACTIVITY OF CLOVE BUD OIL ADMIXED WITH OXAZOLONE IN THE LOCAL LYMPH NODE ASSAY.

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The potential for interaction between contact allergens during the induction phase of skin sensitization has rarely been investigated. Understanding of the potential for additive and/or synergistic effects might prove relevant and inform risk assessment strategies. In order to explore the potential for interactions between chemicals during the acquisition of skin sensitization a series of local lymph node assays (LLNA) were conducted with a well-characterized sample of clove bud oil - an essential oil containing multiple contact sensitizers – alone, and in combination with a constant amount of the strong sensitizer oxazolone. Clove bud oil was tested at five concentrations ranging from 1.0% to 25% w/v in a vehicle of 1:3 ethanol:diethyl phthalate (EtOH:DEP), both with and without 0.005% oxazolone. Two vehicle controls were included, one containing 0.005% oxazolone and one without. The concentration of oxazolone was selected based on a known EC3 value of <0.001% in EtOH:DEP. Stimulation indices (SI) were calculated for each dose level with an SI ≥ 3 compared with the relevant vehicle control being considered positive. Theoretical dose response curves based upon summation of the individual responses to clove oil and the oxazalone containing vehicle controls were also generated. Comparison of the theoretical with experimentally derived dose responses revealed that the combination of clove bud oil and oxazolone had a slightly greater than additive effect, particularly at higher doses. These results are consistent with other reports of additive effects in the LLNA. Further work is needed to determine if these effects translate into increased levels of sensitization to the individual materials.

PS 1516 INDUCTION OF SENSITIZATION AND CYTOTOXICITY TO TRICHLOROETHYLENE IN MICE: ROLE OF METABOLITES.

D. Yufei, L. Haishan, L. Qing, S. Yaofeng, N. Yong, B. Ping and Z. Yuxin. *Key laboratory, National institute for occupational health and poison control, Chinese center for disease control and prevention, Beijing, China.* Sponsor: Z. Yuxin.

Trichloroethylene (TCE) is an important industrial chemical and is widely used. The severe allergic skin damage induced by TCE has been described. The aim of the study was to explore role of metabolites of TCE in the process of allergic skin damage in mice using a combination of *in vivo* and *in vitro* endpoints. Female BALB/c mices pretreated with ethanol or phenobarbital (PB) were administered TCE for 6 weeks, followed by challenge onto the ears to evaluate delayed-type hypersensitivity (DTH) response. After the mice were sacrificed, activities of aspartate aminotransferase and alanine aminotransferase in serum were measured as markers of hepatic damage. The TCE-specific lymphocytes proliferation and cytotoxicity of TCE and its metabolites were determined in cultured spleen lymphocytes exposed to TCE and SKF-525A (CYP450 inhibitor) or aminooxyacetic acid (AOAA, cysteine conjugate β-lyase inhibitor) *in vitro*. The results showed that no DTH response could be observed in 3 groups of mice treated by TCE, TCE+PB and TCE+ethanol. However, a significant proliferation of spleen cells from TCE-treated mice was evidenced following incubation with TCE *in vitro*, it was interesting that this proliferation disappeared when AOAA was added and cytotoxicity of TCE decreased when SKF-525A was added. In mice treated by TCE+PB and TCE+ethanol, no significant proliferation of spleen cells was found in culture with TCE *in vitro*, but hepatoxicity of TCE *in vivo* was promoted by PB and ethanol through increasing the CYP450-dependent oxidation metabolic flux. This study provides an indirect evidence that Glutathion-dependent pathway might play an important role in allergic reaction induced by TCE, and dichlorovnyl thiol-metabolite catalyzed by cysteine conjugate β-lyase is responsible for proliferation of hapten- specific lymphocytes in TCE-sensitized mice. The CYP- dependent oxidation cause cytotoxicity of spleen cells *in vitro*, and hepatotoxicity *in vivo* as well.

PS 1517 INCREASED CELL PROLIFERATION IN SPLEEN AND LYMPH NODES PERIPHERAL TO CONTACT ALLERGEN APPLICATION SITE.

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The local lymph node assay (LLNA) is widely used to identify chemicals that are contact sensitizers. The assay involves dosing mice with the chemical on both ears and pooling the cervical lymph nodes for assessment of lymphocyte proliferation as a marker of sensitization. The present study explored potential reduction in animal usage by dosing one ear with the allergen and the other with vehicle only. The respective draining lymph nodes were processed separately for quantification of cell proliferation. Cell proliferation in axillary and renal nodes, and in the spleen was also assessed. Cross contamination of the chemicals from the dosed ears to other parts of the body via preening was prevented by dosing restrained animals and washing off the residual chemical with saline after 4 hours. Dosing the left ear with 0.02% oxazolone (OX) on unrestrained animals resulted in marked cell proliferation in its draining lymph node (Stimulation index, SI = 12.8) and in the lymph node draining the contra-lateral vehicle dosed ear (SI = 6), and the axillary lymph nodes (SI = 3.3). Increased tritiated thymidine (^{3}H-TdR) incorporation was not observed in the renal lymph nodes (SI = 1.1). Similar stimulation of cells was observed in the lymph node draining the ear contra-lateral to the allergen dosed ear when 30% hexylcinnamaldehyde (HCA) was applied. Increased proliferative activity was observed in non-directly draining lymph nodes of restrained mice demonstrating that these results can not be attributed to cross-contamination of adjacent skin. A significant increase in proliferation of splenocytes was observed. It is concluded that epicutaneous application of a contact allergen, as exemplified by OX and HCA, may induce cell proliferation in the neighboring lymph nodes and spleen indicative of hapten and/or haptenated proteins diffusing through the skin to peripheral nodes and the blood to produce systemic sensitization. Thus the node draining the contra-lateral ear can not be used as a control for application of contact allergen to a single ear in a modified LLNA.

PS 1518 COMPARISON OF GLOVE CONTACT ALLERGEN CONTENT AND CLINICAL PATCH TEST.

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Irritant dermatitis, allergic contact dermatitis (ACD) and urticarial reactions have been associated with chemical rubber accelerators present in latex and nitrile/synthetic rubber gloves. In this study, patients reporting to a dermatology clinic with glove associated ACD were allergy patch tested with the North American Contact Dermatitis Group standard rubber allergen series, medical history taken and asked to identify and supply "problem gloves" which they associated with their ACD and if possible "non-problem gloves". Medical exam, surgical and industrial-type rubber and nitrile gloves from patients were assessed for chemical content including: zinc dithiocarbamates, thiurams, mercaptobenzothiazole (MBT) and its disulfide, dimorpholine, diphenylguanidine, thiourea, and phenylene diamine. Only zinc di-

ethyl - (ZDEC) and zinc dibutyl dithiocarbamate (ZDBC), MBT and dimorpholine were found in glove extracts. Considerable discordance was found between clinical patch test results and glove chemical content. Approximately 37% of patients had no discordance. However, similar percents of patients identified "problem gloves" that did not contain their patch test positive allergen or provided "non-problem gloves" containing the patch test positive allergen. The lowest glove allergen levels associated with a patch test-confirmed ACD were 584, 283 and 590 μg/g glove of ZDEC, ZDBC and MBT, respectively. Discordance between the allergy patch test and glove chemical accelerator content may be in part attributed to both false positive/negative allergy patch test rates and misidentification of problem/non-problem gloves due to the delay between glove usage and clinical ACD manifestation.

PS 1519 TRICHLOROETHYLENE INGESTION ENHANCES PROLIFERATION RATE OF T CELLS AND CYTOKINE PRODUCTION ON MICE.

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Trichloroethylene (TCE) contamination of groundwater has been a social problem because of its toxicity and persistency. We reported that TCE ingestion from drinking water enhanced passive and active cutaneous anaphylaxis (PCA and ACA) reaction on mice. In this study we investigated a mechanism on the enhancement of allergic reaction. We first measured the proliferation rates of the naive mouse splenocytes and lymphocytes exposed to TCE (1 pM- 1 μM) in vitro. The proliferation rates of the splenocytes and lymphocytes were increased by TCE exposure. BALB/c mice were treated with 0.03 mg/L and 3 mg/L TCE dissolved in drinking water for 2 weeks, and immunized twice with ovalbumin (OVA) on the first day and a week after. On the final day of the TCE treatment period, we collected mice spleens and prepared splenocytes. The proliferation rates of splenocytes and cytokine levels of the conditioned medium were measured. The proliferation rates of splenocytes were enhanced by TCE ingestion. IL-2, IL-3, IL-4, IL-10,IFN-γ and TNF-α levels in the conditioned medium of splenocyte increased in TCE treated group. These results show that TCE ingestion enhances T cell proliferation and cytokine production. Therefore, TCE ingestion from drinking water may lead to increase patients of allergic diseases.

PS 1520 MITOGEN-ACTIVATED PROTEIN KINASES CONTROL NRF2 ACCUMULATION IN HUMAN DENDRITIC CELLS IN RESPONSE TO THE CHEMICAL SENSITIZERS.

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Exposure of dendritic cells (DC) to skin contact sensitizers, such as nickel (NiSO4) or dinitrochlorobenzene (DNCB), induce an up-regulation of phenotypic markers and cytokine secretion that are regulated by mitogen-activated protein kinases (MAPKs). Our current hypothesis is that chemical sensitizers generate a chemical stress that is perceived by DCs as a danger signal leading to DC maturation. Among signalling pathways known to be
redox-sensitive, the Keap1/Nrf2 pathway is central for the detection of endogenous or exogenous electrophiles. Briefly, in the absence of electrophile, Keap1 associates with Nrf2 and targets Nrf2 for degradation. In the presence of an electrophilic compound, Keap1 conformation is modified, Nrf2 is released and translocates to the nucleus to act as a transcription factor.
Because sensitizers activate both MAPKs and Nrf2, we address the question whether Nrf2 accumulation by contact sensitizers depends on MAPK pathways. Human monocytes derived DC (Mo-DC) were obtained from peripheral blood and cultured in the presence of GM-CSF and IL-4 for 5 days. Mo-DCs were preatreated with MAPKs inhibitors and then treated with nickel sulfate (500 μM) or DNCB (20 μM). Our results showed that NiSO4 and DNCB induced an accumulation of the Nrf2 protein in Mo-DC. The mRNA expression of target genes of Nrf2, such as *hmox 1* (heme-oxygenase 1) and *nqo1* (NADPH quinone oxydoreductase), were up-regulated after treatment in response to NiSO4 and DNCB. Furthermore, an inhibitor of P38MAPK, SB203580, reduced the accumulation of Nrf2 protein in NiSO4-treated MoDC whereas an ERK inhibitor (PD98059) induced the accumulation of Nrf2 in NiSO4-treated MoDC.
These results suggest that accumulation of Nrf2 in MoDC is controlled by P38MAPK and ERK, two kinases involved in DC maturation. We are currently investigating the role of Nrf2 in DC maturation.

PS 1521 ANALYSING DISCORDANT LOCAL LYMPH NODE ASSAY DATASETS: IMPLICATIONS FOR REACH.

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The local lymph node assay (LLNA) is the assay of choice in European regulatory toxicology. As with other toxicology/sensitisation assays, it has a potential for false results, the anionic surfactant sodium lauryl sulphate (SLS) representing a classic example. In the work reported here, examples of false positives in the LLNA are compared to published "benchmarks" such as SLS. Clear false positives (eg oleic acid) are also contrasted with examples where data interpretation is more challenging. As the LLNA will be applicable to >30,000 chemicals under REACH, and in the light of animal welfare considerations to do no more than the absolute minimum of animal testing, results from a single LLNA often represent the only available data on sensitisation. This reinforces the need to ensure data from this assay are interpreted intelligently, using scientific analysis of results and considering the weight of evidence, before decisions are made on which substances should be classified as representing a skin sensitisation hazard. In chemical classes where the LLNA has been shown to be an inappropriate assay other standardised methods (e.g. the Buehler or Magnusson and Kligman guinea pig tests [OECD 406]) should be employed as the first choice assays.

PS 1522 THE MURINE LOCAL LYMPH NODE ASSAY WITH NON-RADIOACTIVE ALTERNATIVE ENDPOINTS.

A. Karakaya, O. C. Ulker and I. Ates. *Department of Toxicology, Ankara University Faculty of Pharmacy, Ankara, Turkey.* Sponsor: A. Karakaya.

The murine local lymph node assay (LLNA) is a predictive test for detection of contact allergens. The LLNA has been endorsed by the US Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and European Centre for the Validation of Alternative Methods (ECVAM) as a stand-alone method for skin sensitization testing. Although the validity of the LLNA, attention was drawn to two disadvantages; radioactive in vivo measurement of lymph node cell proliferation (3H-thymidine labeling) and possibility of false positive results caused by non-specific cell activation as a result of inflammatory processes in the skin (irritation). In the present study, we investigated the development of non-radioactive endpoint of LLNA based on 5-bromo-2'-deoxyuridine (BrdU)(NR-LLNA) and compared in vivo and ex vivo BrdU incorporation by enzyme-linked immunosorbent assay (ELISA) to improve animal welfare. Female BALB/c mice were treated by the topical application of strong sensitizer 2,4-dinitrochlorobenzene (DNCB) in acetone:olive oil (4:1 v/v) and dimethylsulfoxide at the concentrations of %0.025, %0.05, %0.01, %0.25. Ear thickness was also measured to determine the differentiation index and by this modification it was aimed to determine the proportion of non-specific activation due to irritating properties of test compound. For all concentrations, irritation effect was not observed. At the concentration of %0.05, stimulation index value was found 3 for DNCB. In vivo NR-LLNA and ex vivo NR-LLNA results were good agreement with previous radioactive LLNA data. The study was supported by The Scientific and Technological Council of Turkey, Project number: 107S365

PS 1523 REDUCING THE NUMBER OF ANIMALS IN THE LOCAL LYMPH NODE ASSAY BY USING A COMMON VEHICLE FOR THE TEST MATERIAL AND ALPHA-HEXYLCINNALDEHYDE.

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The Local Lymph Node Assay (LLNA) requires the inclusion of a known sensitizer as a positive control as described in the OPPTS 870.2600 EPA Health Effects Test Guidelines. Historically, a 21% solution of the commercially available alpha-hexylcinnamaldehyde, tech., 85% (HCA, CAS 101-86-0) has been diluted in an acetone-olive oil (4:1 v/v) solution (AOO) and applied to the dorsum of the ears of a group of mice. This approach worked well when the test substance was also soluble

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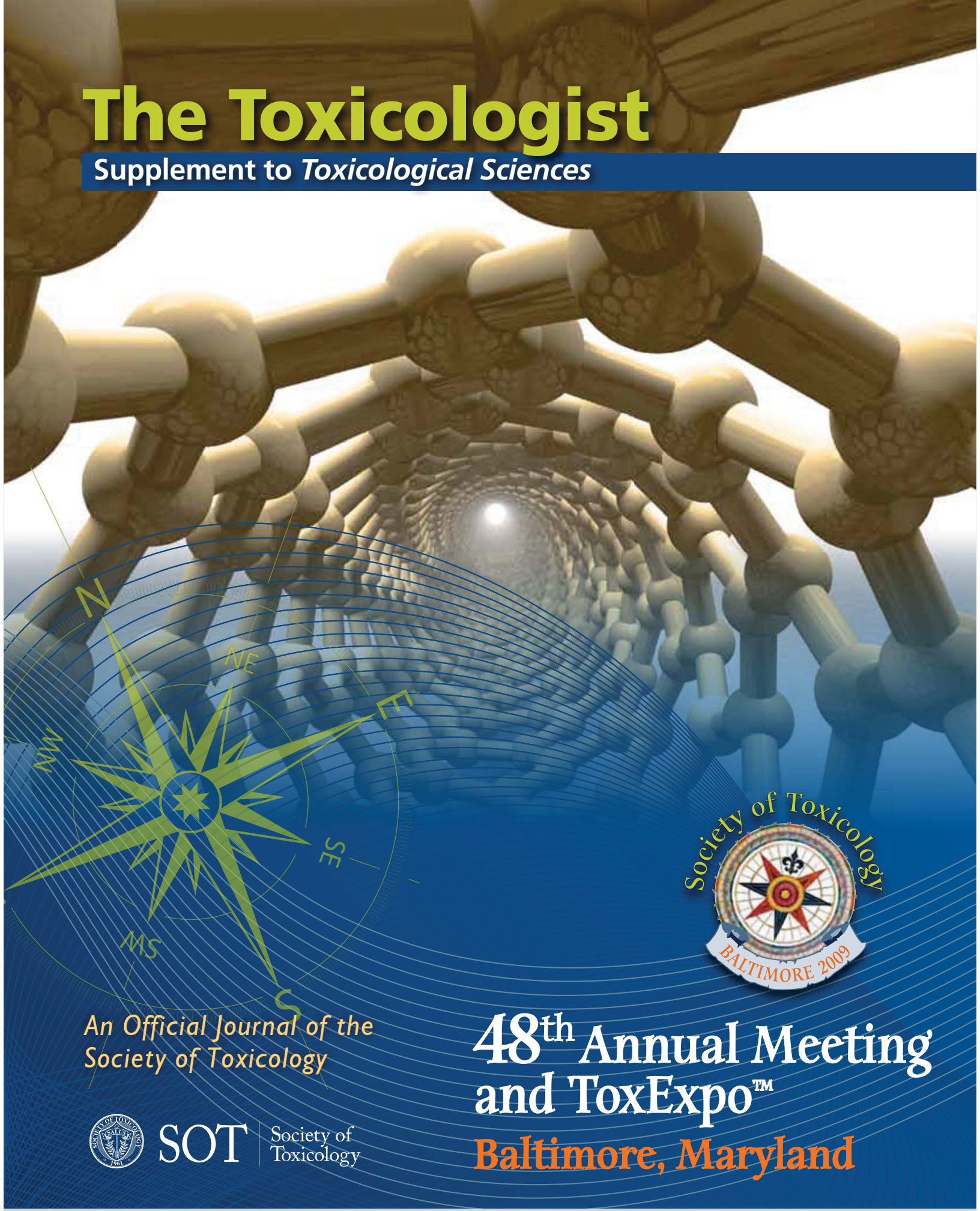

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