UPDATE
National Toxicology Program

AUGUST 2009

Headquartered at the National Institute of Environmental Health Sciences NIH-HHS

Countries Unite to Reduce Animal Use in Product Toxicity Testing

What's Inside:

Article by Robin Mackar, reprinted from *eFACTOR*, July 2009

Participants gathered for a group photo. Shown left to right are Hajime Kojima, Birnbaum, Anklam, Stokes, in uniform, Wind and Blakey. (Photo courtesy of NIH)

Representatives from four international agencies, including the director of the U.S. National Toxicology Program (NTP), signed a memorandum of cooperation on April 27 that could reduce the number of animals required for consumer product safety testing worldwide. The agreement between the United States, Canada, Japan and the European Union will yield globally coordinated scientific recommendations on alternative toxicity testing methods that should speed their adoption in each of these countries, thus reducing the number of animals needed for product safety testing.

"Signing this international agreement demonstrates our commitment to finding and advancing alternatives to animal testing," said Linda Birnbaum, Ph.D., director of the NTP and NIEHS. "This agreement will help us achieve greater efficiency by avoiding duplication of effort and allowing us to leverage limited resources."

Birnbaum signed as the U.S. representative on behalf of the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), one of the national validation organizations participating in the agreement. Others who signed include Elke Anklam, Ph.D., for the European Centre for the Validation of Alternative Methods (ECVAM), David Blakey, D.Phil., for the Environmental Health Science and Research Bureau within Health Canada, and Masahiro Nishijima, Ph.D. for the Japanese Centre for the Validation of Alternative Methods (JaCVAM).

The agreement promotes enhanced international cooperation and coordination on the scientific validation of non- and reduced-animal toxicity testing methods. If the toxicity testing methods are shown to be reproducible based on strong scientific information, and able to accurately identify product related health hazards, the tests are more readily accepted by regulatory agencies.

"The memorandum covers three critical areas of test method evaluation: validation studies, independent scientific peer review meetings and reports, and development of test method recommendations for regulatory consideration," said Marilyn Wind, Ph.D., chair of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and a scientist at the Consumer Product Safety Commission (CPSC).

"This international cooperation will benefit both people and animals," said William Stokes, D.V.M., director of NICEATM and executive director of ICCVAM. Stokes is

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also an assistant surgeon general in the U.S. Public Health Service. “The cooperation will serve an important role in translating research advances into more effective public health prevention tools. It will speed the adoption of new test methods based on advances in science and technology that will provide more accurate predictions of safety or hazard. Animal welfare will also be improved by the national and international acceptance of alternative test methods that reduce, refine, and replace the use of animals.”

Federal agencies are committed to the welfare of animals used in research. All animals used in federally-funded research are protected by laws, regulations and policies to ensure they are used in the smallest number possible and with the greatest commitment to their comfort. ICCVAM is working to promote the development and validation of alternative test methods. Alternative test methods are those that accomplish one or more of the 3Rs – reducing the number of animals used in testing, or refining procedures so animals experience less pain and distress, or replacing animals with non-animal systems.

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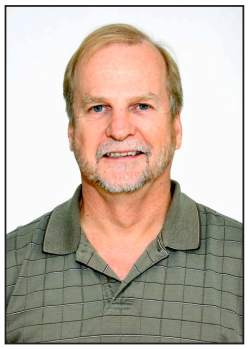
EPA Leader Reports on Progress in Toxicity Testing

Article by Eddy Ball, reprinted from *eFACTOR*, July 2009



Robert Kavlock, Ph.D., visited NIEHS on May 7 to present a status report on the ToxCast™ program he oversees as the director of the National Center for Computational Toxicology (NCCT) at the U.S. Environmental Protection Agency (EPA). EPA is a partner in the five-year Memorandum of Understanding (MOU) signed in 2008 with NIEHS and the National Human Genome Research Institute to improve the evaluation of health risks posed by chemicals found in the environment.

Members of this MOU are informally known as the Tox21 community — for toxicology in the 21st century. This seminar was sponsored by the National Toxicology Program (NTP) Biomolecular Screening Branch, which is responsible for managing the NTP’s High Throughput Screening (HTS) Initiative. The presentation by Kavlock, “ToxCast: Profiling Bioactivity, Identifying Pathways and Predicting Toxicity,” attracted a group of scientists from NIEHS and the NTP, including Linda Birnbaum, Ph.D., director of both organizations.

The goal of ToxCast™ is to develop a high throughput-based program for evaluating the biological targets of environmental chemicals responsible for adverse effects in exposed individuals. EPA needs such a process in order to close the large information gap that exists for thousands of

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Upcoming Events

November 18-19, 2009

NTP Board of Scientific Counselors Technical Reports Review Subcommittee Meeting

NIEHS
111 TW Alexander Drive
Research Triangle Park, NC

December 9-10, 2009

NTP Board of Scientific Counselors Meeting

NIEHS
111 TW Alexander Drive
Research Triangle Park, NC

http://ntp.niehs.nih.gov/go/calendar

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man-made chemicals present in the environment. EPA began planning the ToxCast program in fiscal year (FY) 2005, with a "reduce-to-practice" target date of FY 2012.

As he talked about ToxCast, Kavlock reviewed progress by the NCCT and the other Tox21 partners toward implementing the vision outlined in a 2007 report by the National Research Council on a vision and strategy for toxicity testing in the 21st century. Kavlock also said he was encouraged by the growing cooperation that NCCT is enjoying from manufacturers and drug companies — potentially valuable sources of privileged toxicity data.

According to Kavlock, the toxicology community now acknowledges that by itself the classical model for animal studies is inadequate for the task of assessing the toxicity of the growing number of chemicals in the environment. Animal studies are expensive — costing millions of dollars for a single chemical — time consuming and controversial, he explained. The challenge scientists and regulators face involves developing cost-effective HTS assays to assess biochemical pathways triggered by chemicals, their "biological fingerprints" and "toxicity signatures," that can be used in predicting the potential health effects of exposures.

"We don't rely on a single technology," Kavlock noted as he reviewed the range of assays the program is using. As an example, he pointed to five different methods the program uses for checking whether a chemical can act as an estrogen.

ToxCast examines how each chemical affects a variety of biological processes and compiles the information into a huge database to complement existing animal data. This data will be combined with information on the chemical's structure and behavior in the human body to help quickly classify chemicals based on their potential for human or environmental harm. Phase One of ToxCast has produced data from 320 chemicals, approximately 500 *in vitro* assays and some 100 *in vivo* endpoints, Kavlock said. The data analysis challenge for such a massive amount of data is daunting. "It's like the early days of microarrays," he added. "We have lots of data, and we're looking for the best ways to interpret the observations."

Phase Two of the program, set to start later this year, will include a groundbreaking collaboration with Pfizer, Inc. To help validate the approach for predicting human toxicity, Pfizer has agreed to provide EPA with information on drugs that were found to cause toxicity in clinical trials.

While he and his audience were upbeat about the future of ToxCast and alternative toxicity testing, Kavlock acknowledged the challenges ahead. "The deluge is coming," he said. "We are really on the tip of the iceberg in terms of looking at the data." Kavlock told the audience, "Toxicology is poised to change its stripes." Looking ahead, he referred to the ToxCast Data Analysis Summit May 14–15 at the EPA regional headquarters in Research Triangle Park.

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Expert Panel Reviews Glass Wool Fibers

Article by Robin Mackar, reprinted from *eFACTOR*, July 2009

An expert panel convened in Chapel Hill, N.C. on June 9–10 by the National Toxicology Program (NTP) to review the scientific literature and consider a recommendation by the North American Insulation Manufacturers Association to change the listing status for glass wool fibers in the upcoming 12th Report on Carcinogens (RoC). The expert panel decided to make separate recommendations for different categories of glass wool fibers.

The panel recommended by a unanimous vote that most glass wool fibers should not be classified either as "known to be a human carcinogen" or as "reasonably anticipated to be a human carcinogen," and should be removed from the RoC listing. Glass wool of respirable size is currently listed in the 11th RoC as "reasonably anticipated to be a human carcinogen."

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However, the panel made a distinction for special glass wool fibers of concern, which they defined as longer, thinner, and less soluble fibers. The expert panel recommended that glass fibers with these characteristics —15 micrometers or greater in length with a dissolution rate equal to or less than 100 nanograms per square centimeter per hour — are listed as “reasonably anticipated to be a human carcinogen” in the 12th RoC. The vote was seven to zero with one abstention, with panel members citing sufficient evidence of carcinogenicity in well-conducted animal inhalation studies as the basis for the recommendation.

After receiving oral public comments from a number of groups, the glass wool fibers expert panel carried out an in-depth review of the draft background document and voted unanimously to accept the background document with the panel’s suggested changes. They then discussed the scientific information for glass wool fibers, applied the RoC listing criteria to the body of evidence, and made a recommendation for listing status in the RoC.

Glass wool refers to fine glass fibers forming a mass resembling wool and is most commonly used for insulation and filtration. There are two categories of glass wool based upon commercial application — insulation glass wool, which is now among the most extensively used insulating material worldwide, and special-purpose fibers, which are used in special applications and make a much up smaller fraction of the market. There are differences in the chemical compositions and physical characteristics of glass fibers, which may influence the toxicology and potential carcinogenicity of different fibers.

Fibers have also been examined based upon other characteristics including persistence, retention and clearance rates, and durability. The European Union and Germany have established criteria for labeling and classifying synthetic vitreous fibers based on their potential human health hazard.

Next, the NTP will solicit public comment on the expert panel’s listing recommendation and scientific justification through the Federal Register and finalize the background document, taking into consideration the panel’s recommended edits and public comments. Afterwards, the agency will convene two independent review groups who will also apply the RoC listing criteria and make a recommendation for listing status in the RoC.

Information about this meeting and the review of wool glass fibers or any other RoC nominated chemical is available online.

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NTP Board of Scientific Counselors

The NTP Board of Scientific Counselors (BSC) will also meet on December 9-10, 2009, at the NIEHS. As plans for this meeting are developed they will posted on the NTP web site or can be obtained by contacting the Executive Secretary, Dr. Barbara Shane. This meeting is open to the public and public comment, both written and oral, is welcome on any agenda topic.

NTP Board of Scientific Counselors Technical Reports Review Subcommittee

The NTP Board of Scientific Counselors Technical Reports Review Subcommittee is scheduled to meet on November 18-19, 2009, at the NIEHS to peer review the findings and conclusions from 7 draft NTP Technical Reports performed in conventional rats and mice, one draft report on studies in a genetically modified mouse model, and one draft report on phototoxicity studies conducted in SKH-1 mice.

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The draft reports tentatively scheduled for review are:

GMM 14	AIDS therapeutics in p53+/- mouse
TR 536	Bis(2-chloroethoxy)methane
TR 561	AIDS therapeutics (transplacental dosing)
TR 563	Pulegone
TR 564	1-Bromopropane
TR 565	Milk thistle extract
TR 566	Diethylamine
TR 567	Ginseng
TR 568	Phototoxicity study of retinyl palmitate

Details about this meeting will be announced in the Federal Register and posted on the NTP web site (http://ntp.niehs.nih.gov/go/15833) or can be obtained by contacting the Executive Secretary, Dr. Barbara Shane. This meeting is open to the public and public comment, both written and oral, is welcome on any report.

Contact Information: : Dr. Barbara Shane, Executive Secretary, NTP Office of Liaison, Policy, and Review, NIH/NIEHS, P.O. Box 12233, MD K2-03, Research Triangle Park, North Carolina 27709; T: (919) 541-4253; FAX: (919) 541-0295; shane@niehs.nih.gov

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NTP Testing Program

Request for Study Nominations

With a broad mandate to provide toxicological characterizations for chemicals and other substances of public health concern, the NTP accepts nominations for new toxicological studies at any time. Labor unions, academic scientists, federal and state agencies, industry, and the general public are welcome to make nominations for specific substances or for general issues related to potential human health hazards of occupational or environmental exposures. As available, a rationale for study should accompany the nomination along with background information describing sources of exposure and possible adverse health effects or concerns associated with exposure, the chemical name and the Chemical Abstract Service (CAS) registry number. Details about the nomination review and selection process are available on the NTP web site (http://ntp.niehs.nih.gov, select *Nominations to the Testing Program* under the heading Testing Information) or by contacting the NTP Office of Nomination and Selection (contact information below).

Current areas of focus in the NTP's testing program include potential hazards associated with radiofrequency radiation from cellular phones, metals, nanoscale materials, perfluorinated compounds, herbal dietary supplements, photoactive chemicals, brominated flame retardants, certain complex occupational exposures, dioxin-like compounds, contaminants of finished drinking water, and endocrine-active substances.

All nominations undergo several levels of review before being selected by the NTP for study. These steps of review help to ensure that the NTP's testing program addresses toxicological concerns pertinent to all areas of public health and helps maintain balance among the types of substances and issues evaluated. Studies are initiated on selected nominations as time and resources permit.

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Study Nominations Currently in Review

The six new study nominations briefly described below were reviewed by the NTP Board of Scientific Counselors (BSC) at a public meeting on July 23-24, 2009. Supporting documents and any public comments received for these nominations are available on the NTP web site at http://ntp.niehs.nih.gov/go/nom; select Nominations in Review - Current.

Information related to the July 23-24 meeting, including draft research concepts for these new nominations, is available on the NTP web site at (http://ntp.niehs.nih.gov/go/165). Questions or comments on any of the new study nominations should be directed to Dr. Scott Masten (contact information below).

- Alkylanilines: a class of aromatic amines used as industrial intermediates and also found in tobacco smoke; recommended for initial toxicological characterization
- 1-Chloro-4-(trifluoromethyl)benzene: a high production volume chemical used for making herbicides and as a solvent; recommended for comprehensive toxicological characterization including developmental and reproductive toxicity and chronic toxicity/carcinogenicity studies
- Deoxynivalenol: a mycotoxin widely occurring as a contaminant in various foods; recommended for comprehensive toxicological characterization including reproductive toxicity and chronic toxicity/carcinogenicity studies
- Dong quai: a widely available herbal dietary supplement touted as a treatment for female reproductive problems; recommended for comprehensive toxicological characterization including phototoxicity studies
- Indium tin oxide: a high volume industrial chemical primarily used in coatings for glass and plastic panel displays; recommended for comprehensive toxicological characterization
- Tris(4-chlorophenyl)methane and tris(4-chlorophenyl)methanol: an environmentally persistent compound of unknown source found in the marine food web; recommended for initial toxicological characterization

Contact Information: Dr. Scott A. Masten, Director, NTP Office of Nomination and Selection, NIH/NIEHS, P.O. Box 12233, MD K2-02, Research Triangle Park, North Carolina 27709; T: (919) 541-5710; FAX: (919) 541-3647; masten@niehs.nih.gov

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New Search Page Added To The NTP Website

The NTP's testing program has added a new web page, http://ntp.niehs.nih.gov/go/nom-search, where users can search to learn about testing nominations. Information on a particular substance is just a click away. This new search will return information about the nomination including its status, nominator, and nomination date. This page should serve as a useful resource for finding current information on the history and status of nominations.

Details about the NTP nomination review and selection process along with an online nomination form are available on the NTP web site at http://ntp.niehs.nih.gov/go/nom.

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Report on Carcinogens Center

Scientific Review of Formaldehyde Planned for Fall 2009

The expert panel meeting for the scientific review of formaldehyde is tentatively planned for Fall 2009. Formaldehyde is a high-production chemical with a wide array of uses and was selected for review for the 12th Report on Carcinogens (RoC). The predominant use of formaldehyde in the United States is in the production of industrial resins and as a chemical intermediate. It is also used as a disinfectant and preservative and in numerous consumer products and is off-gassed from formaldehyde-containing materials such as carpets, paint, and insulation. Formaldehyde (gas) is currently listed in the 11th RoC as reasonably anticipated to be a human carcinogen. Further information about this expert panel meeting will be announced in a Federal Register notice and on the RoC website by early September.

Contact Information: Dr. Ruth M. Lunn, NTP Report on Carcinogens Center, NIH/NIEHS, P.O. Box 12233, MD K2-14, Research Triangle Park, NC 27709; T: (919) 316-4637; FAX: (919) 541-0144; lunn@niehs.nih.gov

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NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Federal Agencies Accept ICCVAM Recommendations on *In Vitro* Pyrogenicity Test Methods

NICEATM, on behalf of Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), forwarded ICCVAM recommendations on five *in vitro* test methods proposed for assessing potential pyrogenicity of pharmaceuticals and other products to Federal agencies in November 2008. All agencies accepted or endorsed the ICCVAM recommendations. The agencies' responses and other information about the test method evaluation are posted on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/pyrogen/pyrogen.htm

These test methods can now be considered prior to conducting *in vivo* pyrogenicity testing, and can be used where determined appropriate for specific testing situations. The availability of these test methods may reduce the number of animals required for pyrogenicity testing.

Panel Reviews Alternative Methods for Ocular Safety Assessments

NICEATM and ICCVAM convened an international, independent panel on May 19-21 at Consumer Product Safety Commission Headquarters in Bethesda, MD to evaluate alternative test methods and approaches that may further reduce and refine the use of animals for ocular safety testing. The panel included 22 scientists from six countries and was chaired by Dr. Wallace Hayes from the Harvard School of Public Health.

The panel reviewed the current validation status of several proposed alternative test methods and testing approaches according to established Federal and international criteria. The panel also commented on draft ICCVAM recommendations regarding the usefulness and limitations of each proposed test method and approach. They also reviewed the appropriateness of refinements to the *in vivo* rabbit eye test including (1) routine use of pain relieving drugs and (2) humane endpoints that could be used to terminate studies early. The panel endorsed proposed recommendations by the ICCVAM that are expected to improve the well-being and reduce the numbers of animals used to determine if consumer products and other chemicals can cause eye injuries.

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The panel's conclusions and recommendations include:

- Topical anesthetics and systemic analgesics should routinely be used prior to any *in vivo* ocular irritancy testing. The panel recommended an enhanced protocol of specific pain-relieving drugs and schedule of administration to effectively avoid or minimize discomfort.
- Proposed non-animal testing strategies using three *in vitro* test methods to assess the eye irritation potential of antimicrobial cleaning products for EPA ocular hazard classification and labeling purposes appear promising. The panel recommended that studies to further characterize the *in vitro* test methods and testing strategies should be designed in coordination with ICCVAM.
- Two *in vitro* test methods for identifying ocular irritants could be used in limited circumstances as screening tests to identify some products and substances that would not require hazard labeling for eye irritation.

The full report is available on the NICEATM-ICCVAM website and public comments are invited. Public comments can be submitted via e-mail to niceatm@niehs.nih.gov or directly at the website http://iccvam.niehs.nih.gov/contact/FR_pubcomment.htm. The deadline for comments is August 28, 2009.

ICCVAM has contributed to the approval or endorsement of 27 alternative safety-testing methods by Federal regulatory agencies since its establishment in 1997. Appropriate use of these test methods can significantly reduce animal use and improve animal welfare. ICCVAM has also identified critical research, development, and validation efforts needed to further advance numerous other alternative methods.

ICCVAM will consider the report along with all comments received from the public and from its scientific advisory committee as it prepares final test method recommendations that will be forwarded to Federal agencies for their consideration later in 2009. These recommendations, if adopted by Federal agencies, are expected to further reduce animal use for ocular safety testing, and may potentially eliminate animal pain and distress in situations where animal testing is still required.

Documents reviewed by the panel and other information can be found on the NICEATM-ICCVAM website at: http://iccvam.niehs.nih.gov/methods/ocutox/PeerPanel09.htm.

Contact Information: Dr. William S. Stokes, Director, NICEATM, NIH/NIEHS, P.O. Box 12233, MD K2-16, Research Triangle Park, NC 27709; T: (919) 541-2384; FAX: (919) 541-0947; niceatm@niehs.nih.gov

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NTP Publications January - March 2009

Publications from NTP Staff at NIEHS, NIOSH, and NCTR are listed below. Access to the journal may require a subscription.

Antonini, JM, Roberts, JR, Stone, S, Chen, BT, Schwegler-Berry, D and Frazer, DG (2009). **"Short-term inhalation exposure to mild steel welding fume had no effect on lung inflammation and injury but did alter defense responses to bacteria in rats."** *Inhal Toxicol* 21(3): 182-92.
PubMed: http://www.ncbi.nlm.nih.gov/pubmed/18925477
DOI: http://dx.doi.org/10.1080/08958370802360661

Apopa, PL, Qian, Y, Shao, R, Guo, NL, Schwegler-Berry, D, Pacurari, M, Porter, D, Shi, X, Vallyathan, V, Castranova, V and Flynn, DC (2009). **"Iron oxide nanoparticles induce human microvascular endothelial cell permeability through reactive oxygen species production and microtubule remodeling."** *Part Fibre Toxicol* 6:1.
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Barbero, AM and Frasch, HF (2009). **"Pig and guinea pig skin as surrogates for human *in vitro* penetration studies: A quantitative review."** *Toxicology* in Vitro 23(1): 1-13.
PubMed: http://www.ncbi.nlm.nih.gov/pubmed/19013230
DOI: http://dx.doi.org/10.1016/j.tiv.2008.10.008

Bienek, DR, Loomis, LJ and Biagini, RE (2009). **"The anthrax vaccine No new tricks for an old dog."** *Human Vaccines* 5(3): 184-189.
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DOI: Not Available

Blachere, FM, Lindsley, WG, Pearce, TA, Anderson, SE, Fisher, M, Khakoo, R, Meade, BJ, Lander, O, Davis, S, Thewlis, RE, Celik, I, Chen, BT and Beezhold, DH (2009). **"Measurement of Airborne Influenza Virus in a Hospital Emergency Department."** *Clin Infect Dis* epub ahead of print
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DOI: http://dx.doi.org/10.1016/j.neuro.2008.10.011

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PubMed: http://www.ncbi.nlm.nih.gov/pubmed/18368011
DOI: http://dx.doi.org/10.1038/jes.2008.18

Bucher, JR (2009). **"Bisphenol A: Where to Now?"** *Environmental Health Perspectives* 117(3): A96-A97.
PubMed: http://www.ncbi.nlm.nih.gov/pubmed/19337497
DOI: http://dx.doi.org/10.1289/ehp.12492

Cao, Y, Calafat, AM, Doerge, DR, Umbach, DM, Bernbaum, JC, Twaddle, NC, Ye, XY and Rogan, WJ (2009). **"Isoflavones in urine, saliva, and blood of infants: data from a pilot study on the estrogenic activity of soy formula."** *Journal of Exposure Science and Environmental Epidemiology* 19(2): 223-234.
PubMed: http://www.ncbi.nlm.nih.gov/pubmed/18665197
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Chang, QS, Zhang, YD, Beezhold, KJ, Bhatia, D, Zhao, HW, Chen, JG, Castranova, V, Shi, XL and Chen, F (2009). **"Sustained JNK1 activation is associated with altered histone H3 methylations in human liver cancer."** *Journal of Hepatology* 50(2): 323-333.
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Cheever, KL, Marlow, KL, B'Hymer, C, Hanley, KW and Lynch, DW (2009). **"Development of an HPLC-MS procedure for the quantification of N-acetyl-S-(n-propyl)-l-cysteine, the major urinary metabolite of 1-bromopropane in human urine."** *J Chromatogr B Analyt Technol Biomed Life Sci* 877(8-9): 827-32.
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DOI: http://dx.doi.org/10.1016/j.jchromb.2009.02.010

Chen, F and Castranova, V (2009). **"Beyond apoptosis of JNK1 in liver cancer."** *Cell Cycle* 8(8): 1145-1147.
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PubMed: http://www.ncbi.nlm.nih.gov/pubmed/19384566
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Chen, HF, Su, PH and Zhu, YM (2009). **"Morphology determination of multi-needle bipolar corona discharge by OES."** *Guang Pu Xue Yu Guang Pu Fen Xi/Spectroscopy and Spectral Analysis* 29(1): 24-27.
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Cheng, Y, Wright, SH, Hooth, MJ and Sipes, IG (2009). **"Characterization of the Disposition and Toxicokinetics of N-Butylpyridinium Chloride in Male F-344 Rats and Female B6C3F1 Mice and Its Transport by Organic Cation Transporter 2."** *Drug Metabolism and Disposition* 37(4): 909-916.
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D'Aloisio, AA, Schroeder, JC, North, KE, Poole, C, West, SL, Travlos, GS and Baird, DD (2009). **"IGF-I and IGFBP-3 Polymorphisms in Relation to Circulating Levels among African American and Caucasian Women."** *Cancer Epidemiology Biomarkers & Prevention* 18(3): 954-966.
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