

## Most cited articles in the Archives of Toxicology: the debate about possibilities and limitations of in vitro toxicity tests and replacement of in vivo studies

H. M. Bolt · J. G. Hengstler

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Every year the Archives of Toxicology celebrate the most cited authors (Table 1). As expected apoptosis, metal toxicity, oxidative stress and regulatory toxicology represented hot topics. The editors would like to highlight two outstanding articles, written by O'Brien et al. (2006) and a review by Greim et al. (2006), because both articles demonstrate important aspects of the current debate about replacement of in vivo studies by in vitro tests. O'Brien and colleagues performed a large validation study about prediction of human hepatotoxicity by a human hepatoma cell line in vitro. Human hepatocytes and also hepatocyte cell lines are frequently used for in vitro studies of toxicity, drug metabolism and enzyme induction (Hewitt et al. 2007; Hengstler et al. 2000; Brulport et al. 2007). Many examples of excellent correlations between in vitro data with hepatocytes and the in vivo situation have been published (Gebhardt et al. 2003; Hengstler et al. 2000; Carmo et al. 2005). However, well-designed validation studies including sufficient numbers of substances are rare. O'Brien and colleagues used a standardized in vitro test with human HepG2 cells and analyzed a well-chosen battery of endpoints. The authors studied 243 compounds with varying degree of toxicity. Overall human toxicity potential was detected with 80% sensitivity and 90% specificity. This is an important study, and also the most cited article published in the Archives between 2006 and 2007. Considering the limitations of an in vitro system based on HepG2 cells, such as incomplete metabolic competence or toxicities mediated by other cell types than hepatocytes, the result is encouraging.

Without doubt well-validated in vitro tests, such as the human hepatocyte screening assay of O'Brien et al. can be used to identify substances with an increased potential to cause specific toxic effects, such as hepatotoxicity in the case of O'Brien's test strategy. This is indeed an important progress. However, two major limitations remain. First, an in vitro test using one particular primary cell type, such as the hepatocytes or a hepatocyte cell line, may have the potential to predict liver toxicity but not toxicity to other organs (which was of course not intended by the authors). Second, at the present state of the art in vitro studies only allow screening for specific hazardous properties but information about in vivo dose–response relationships and identification of NOELS cannot be obtained. This is where the review of Helmut Greim and further recognized European Toxicologists comes in (Greim et al. 2006). The authors discuss one of the problematic aspects of the new European chemicals legislation REACH according to which repeated dose studies may only be required for substances produced beyond 100 t/year. This means that most of the substances will not be studied by repeated dose tests such as 28- and 90-day studies. The authors criticize that in the absence of such information the hazard identification will be incomplete. Alternative in vitro studies alone will not identify all relevant endpoints of in vivo toxicity and can not identify thresholds and NOELS. Therefore, the authors criticize that a premature replacement of in vivo tests primarily considers cost reduction and protection of animals, whereas the protection of human health and the environment are secondary. The editors feel that this is an important contribution to the recent debate. It also leads to the discussion to which extent and when we will be able to predict NOELS only by in vitro tests. The latter has recently been discussed in this journal by Li-ienblum et al. (2008) and large EU projects focus on his

H. M. Bolt · J. G. Hengstler (✉)  
Institut für Arbeitsphysiologie an der Universität Dortmund,  
Leibniz Research Centre for Working Environment and Human  
Factors (IfADo), Ardeystrasse 67, 44139 Dortmund, Germany  
e-mail: hengstler@ifado.de

**Table 1** The 20 most cited articles in the Archives of Toxicology in 2006 and 2007

No.	Author	Take home message
1	O'Brien et al. 2006	Human hepatocytes in vitro predict human hepatotoxicity potential with high sensitivity and specificity in a large validation study
2	Stefanidou et al. 2006	Physiological supplementation of zinc reduces risk of age-related diseases
3	Hwang et al. 2006	The plant alkaloid berberine decreases Bcl-xL expression and causes apoptosis in hepatoma cells
4	Boettcher et al. 2006	The metabolic profile of acrylamide in humans is more similar to that of rats than of mice
5	Reistad et al. 2006	The flame retardants DE-71 a pentabrominated diphenyl ether and hexabromocyclododecane have a neurotoxic potential
6	Barbosa et al. 2006a	Lead exposure was associated with decreased nitric oxide formation which may explain lead associated increased cardiovascular risk
7	Schrickx et al. 2006	The mycotoxin ochratoxin A is secreted by the ATP-dependent membrane transporters MRP2 and BCRP
8	Zhan et al. 2006	Fluoride causes lipid oxidation and apoptosis in livers of pigs
9	Li et al. 2006	Aristolochic acid I causes DNA strand breaks and cell cycle arrest in renal tubular epithelial cells which may explain Aristolochic acid associated nephrotoxicity
10	Wu et al. 2006	Cigarette smoke exposure induces apoptosis in lungs of rats
11	Greim et al. 2006	According to REACH repeated dose studies (28-day and 90-day studies) are only required beyond 100 t/a. This is criticized, because alternative in vitro studies do not allow identification of all relevant hazardous properties and do not give adequate information on NOELS
12	Kim et al. 2007	The metal transporters DMT1 and MTP1 play a key role in tissue cadmium accumulation
13	Nampoothiri et al. 2007	Cadmium and lead accumulate in the ovary of rats and induce lipid peroxidation
14	M'Bemba-Meka et al. 2007	Intracellular calcium is critical for nickel carbonate hydroxide induced genotoxicity
15	Raulf-Heimsoth et al. 2007	Aerosols of bitumen cause irritative airway effects in exposed workers, as evidenced by increased neutrophil counts and cytokine sputum concentrations in highly exposed individuals
16	Barbosa et al. 2006b	Salivary lead is not an adequate biomarker for lead exposure
17	Moto et al. 2006	The non-genotoxic carcinogen dicyclamil induces oxidative stress in livers of mice
18	Myhrer et al. 2006	A new microinfusion technique allows a more detailed examination of anticonvulsant properties of drugs
19	Yang et al. 2006	Decane, undecane and dodecane are the major toxic hydrocarbons of jet fuels
20	Montenegro et al. 2006	A polymorphism of the delta-aminolevulinic acid dehydratase gene (ALAD 1-2/2-2) identifies subjects with increased susceptibility to lead exposure

task. In principle, all individual problems associated with in vitro in vivo extrapolation can be solved by pharmacokinetic modelling. However, the difficulty is complexity and the necessity to take into account all relevant factors of influence. In some future it may be possible to predict in vivo toxicity only by in vitro tests. We can not say whether this will be achieved in 15 or in 50 years. However, we can say for sure that this goal can not be achieved within 5 years. In the meantime as outlined by Greim and colleagues we have two possibilities (Greim et al. 2006): Continue the existing methods until reliable in vitro techniques are available or reduce the quality of hazard and risk evaluation which will probably be associated with adverse consequences for human health.

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