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Computers and Humans in the SAR Assessment of Health Hazards

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Computers and Humans in the SAR Assessment of Health Hazards[#]

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Abstract

The SAR-generated toxicological profile of gabapentin was subjected to human scrutiny and compared to experimentally derived data. When both experimental data and SAR projections were available, there was excellent concurrence. The SAR projections together with analyses of their basis indicate that GBP is not likely to present a carcinogenic hazard by mechanisms relevant to humans. The analyses emphasize the importance of human expertise in interpreting and accepting SAR predictions of toxicological effects.

Keywords. Gabapentin; toxicity; SAR; hazard identification.

1 INTRODUCTION

SAR approaches to identify chemicals that present potential health hazards are gaining acceptance in the research, product development and regulatory phases [1–8]. In order to retain this recognition, especially in the regulatory arena, SAR paradigms must meet rigorous validation, transparency and statistical criteria [2–8]. Our laboratory has been involved in substructure-based SAR development, validation and characterization processes [9]. We have stressed the importance of human expertise in the generation and subsequent interpretation of SAR projections [10,11]. In the present set of analyses we examine this SAR–human interaction in the health hazard identification process. We scrutinize a therapeutic agent for which there is already a substantial body of toxicological and clinical data. This will enable us to (a) compare experimental findings with SAR predictions, (b) delve into the mechanistic significance of the previous toxicological findings and (c) extend further the toxicological profile of the test agent. Gabapentin (GBP, 1-(aminomethyl)cyclohexaneacetic acid, CAS No. 60142–96–3, Figure 1A) is one of the newer anticonvulsants used in the treatment of epilepsy, cocaine-induced seizures and pain [12–16].

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Given its wide usage, GBP's side effects have been evaluated. GBP is generally well-tolerated and of low acute toxicity, although individual instances of toxicity have been reported especially in patients receiving GBP in combination with other antiepileptic drugs [13,17–21]. The study of the potential toxicity of GBP is facilitated by the fact it is not metabolized in mammals [15,22].

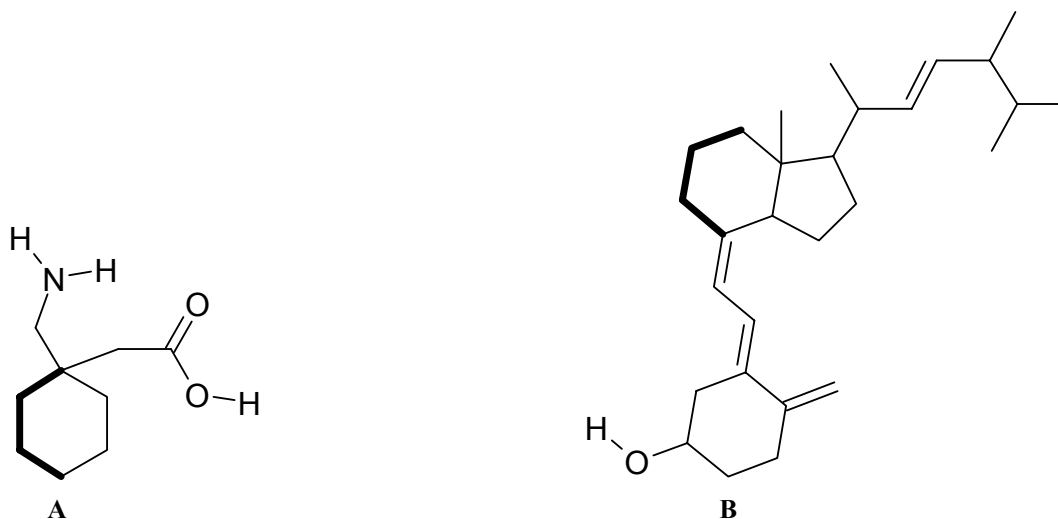


Figure 1. The SAR program indicates a slight possibility that GBP (A) might be a mouse carcinogen based upon the presence of the putative toxicophore shown in bold. That putative toxicophore, however, is derived from a *single* chemical, the carcinogen calciferol (B). The latter, however, is present in a very different environment than GBP. Based upon that structural difference and the fact that the prediction is derived from a single chemical, the prediction of carcinogenicity in mice was deemed irrelevant.

2 MATERIALS AND METHODS

2.1 SAR Modeling

The MULTICASE SAR program was used. The algorithms employed within this program have been described previously [9,23,24]. Input is in the form of a database, composed of a set of chemical structures of interest and their respective experimentally determined biological activities (quantitative or qualitative). The program provides a means to identify descriptors consisting of molecular fragments, ranging from two to ten heavy atoms along with their associated hydrogens, which account for the biological activity of the compounds under study. The molecular fragments are generated as a result of breaking down each individual chemical structure within a data base into its constituent parts. Each fragment is “labeled” with respect to its origin within active or inactive compounds. Fragments of relevance are those that exhibit statistically significant non-random distribution among the active and inactive classes of compounds. In addition to utilizing molecular fragments, MULTICASE identifies relevant two-dimensional distances between atoms within a chemical structure.

MULTICASE utilizes the set of statistically significant descriptors (fragment and/or distance) to find a descriptor (biophores/toxicophores) that has the highest probability of being responsible for the observed biological activity. Compounds within the database containing the primary biophore/toxicophore are removed from the analysis and subsequent biophores are selected that explain the activity of the remaining compounds. This iterative process of selection is continued until either all of the active compounds are accounted for, or no statistically significant descriptors remain. The presence of toxicophores determines the likelihood that a compound exhibits activity. Predictions made on compounds submitted for SAR analysis consist of the identification of the toxicophores responsible for activity and the percent probability of the compound being biologically active due to such occurrences. A compound is presumed to be inactive if it contains no toxicophores.

MULTICASE also attempts to derive a "local" QSAR within each group of compounds containing a particular toxicophore. This will identify molecular features that control the degree of activity. These features, termed modulators, are selected from the pool of molecular fragments, distance descriptors, calculated electronic indices (molecular orbital energies, charge densities) and calculated transport parameters (octanol/water partition coefficient, water solubility). The local QSARs are utilized to predict the potency of chemicals containing the specific toxicophore. The SAR model's performance, *i.e.* the predictive power (Q^2), was determined by its ability to predict chemicals external to the dataset used to generate the model [6,8,25–27].

2.2 SAR Models

The SAR models used were derived using the MULTICASE SAR expert system [9,23,24]. The validated models used herein have been characterized with respect to their ability to predict the activity of chemicals external to the model [9,28,29]. The models, for the most part, have been described previously: inhibition of gap junctional intercellular communication (iGJIC) [30]; structural-alerts for DNA reactivity [31,32]; induction of mutations at the *tk*^{+/-} locus of cultured mouse lymphoma cells [33]; induction of sister chromatid exchanges (SCE) and chromosomal aberrations in cultured CHO cells [34], of micronuclei [35] and SCE *in vivo* [36]; of yeast malsegregations [37]; perturbation of tubulin polymerization [38]; binding to the *Ah* receptor [39]; developmental toxicity in mice [40], rats [40], hamsters [41] and rabbits [40]. Models of developmental toxicity in humans were based upon two independent datasets [40,42]. Mutagenicity in *Salmonella* [43,44]; error-prone DNA repair (*i.e.* the SOS Chromotest) [45,46]; carcinogenicity in rodents (a combination of the results of bioassays conducted by the U.S. National Toxicology Program (NTP) [47] and of those included in the Carcinogenic Potency Data Base (CPDB) [48]) were also described earlier. The results of the predictions using the SAR models were combined into a single probability [49,50]. SAR models of the induction of unscheduled DNA synthesis in rat hepatocytes [51], cell toxicity for murine Balb 3T3 cells (clonal assay) and human HeLa cells (dye

retention assay) were also described previously [52,53]. Cell toxicity was defined as IC₅₀ values < 1μM and 7mM for Balb3T3 and HeLa cells, respectively.

The SAR model of α₂μ-globulin associated nephropathy in male rats was based upon data kindly provided by Dr. L. D. Lehman-McKeenan (Procter and Gamble Co.). The SAR models for the inhibition of human cytochrome P4502D6 (cyp2D6) [54], cell transformation [52] and umu/SOS DNA repair [55] were based upon previously described data. Acute toxicity in rats was defined as an LD₅₀ ≤ 7.3mmol/kg. The SAR model was based on data of orally administered chemicals extracted from the Registry of Toxic Effects of Chemical Substances [56]. SAR models of the maximum tolerated dose (MTD) in mice and rats were based upon chemicals tested in the NTP cancer bioassays. MTD values were expressed as gavage equivalents. MTD values below 0.9 and 1.8 mmol/kg/day were taken as indications of systemic toxicity in mice and rats, respectively [57].

3 RESULTS AND DISCUSSION

The relatively low acute toxicity of GBP together with its usage indications results in a prolonged therapeutic regimen [58]. Accordingly, in assessing the potential liabilities of GBP therapy, one must consider not only its acute toxicological effects but also the possible unwanted effects resulting from chronic exposure. These include an evaluation of its possible carcinogenicity, developmental toxicity and associated effects.

In a series of rodent carcinogenicity studies, it was shown that while GBP was non-carcinogenic to male and female mice and to female rats, it induced pancreatic acinar cell neoplasias in male Wistar rats [59]. These tumors were seen only at high GBP doses, they did not metastasize and they arose late in the bioassay [59].

It is generally recognized that rodent carcinogens that present a carcinogenic risk to humans are those that are “genotoxic”, *i.e.* they induce mutations, DNA damage and possibly clastogenicity. In fact the vast majority of recognized human carcinogens exhibit such properties [60–63]. The other type of recognized human carcinogens are hormones (*e.g.* β-estradiol) which act by a receptor-mediated mechanism [64].

Based upon a battery of validated and characterized SAR models [9], GBP is shown to lack a “structural alert” for DNA reactivity (Table 1, Analysis No. 1), the potential to induce mutations in prokaryotes (*Salmonella*) (Table 1, Analysis No. 2) and eukaryotes (tk^{+/-} locus of cultured mouse lymphoma cells) (Table 1, Analysis No. 6), DNA-damage in prokaryotes (Table 1, Analyses Nos. 3 and 4) and eukaryotes (Table 1, Analysis No. 5) as well as clastogenicity *in vitro* (Table 1, Analyses Nos. 9 and 10) and *in vivo* (Table 1, Analyses Nos. 7 and 8). The results confirm the reported lack of mutagenicity, clastogenicity and induction of micronuclei [65–67]. Altogether, these results

strongly suggest that GBP lacks DNA damaging properties and hence its potential to cause cancers in humans by a genotoxic mechanism is very low. In fact, based upon the SAR projections (Table 1) and the known predictive parameters (*i.e.* sensitivities and specificities) of some of these models (“structural alerts” for DNA reactivity, mutagenicity in *Salmonella*, mutagenicity at the *tk*^{+/-} locus of mouse lymphoma cells, of sister chromatid exchanges and chromosomal aberration *in vitro*, and the *in vivo* induction of micronuclei for carcinogenicity) and applying Bayes’ theorem, assuming a prior probability of 0.5, it is found that the probability that GBP is a genotoxicant is extremely low, *i.e.* 0.045) [68].

Table 1. Predicted Toxicological Profile of Gabapentin

No.	System	Probability of activity %		Conclusion
1	Structure Alerts:	0		I
2	Salmonella Mutagenicity:	0		I
3	Error-prone DNA repair	0		I
4	umu/SOS Repair	0		I
5	Unscheduled DNA Synthesis in vitro	0		I
6	Mutations in Mouse Lymphoma	0		I
7	Induction of Micronuclei in vivo	0		I
8	Sister Chromatic Exchanges in vivo	0		I
9	Sister Chromatic Exchanges in vitro	0		I
10	Chromosomal Aberrations in vitro	0		I
11	Yeast Malsegregation	0		I
12	Inhibition GJIC	75		A
13	Cell Transformation	0		I
14	Inhibition of Tubulin Polymerization	0		I
15	Rat MTD	0		I(>1.8 mmol/kg)
16	Mouse MTD	0		I(>0.9 mmol/kg)
17	Carcinogenicity: Rodent-NTP	0		I
18	Carcinogenicity: Rats-NTP	0		I
19	Carcinogenicity: Mice-NTP	0		I
20	Carcinogenicity: Rodent-CPDB	0		I
21	Carcinogenicity: Rats-CPDB	0		I
22	Carcinogenicity: Mice-CPDB	67		I*
23	Carcinogenicity: Overall			Non-Genotoxic Non-Carcinogen*
24	Binding to Ah Receptor	0		I
25	Inhibition Human cyp2D6	100		A
26	Nephrotoxicity: Male Rats(α 2 μ)	100		I*
27	Developmental Toxicity: Hamster	0		I
28	Developmental Toxicity: Mouse	0		I
29	Developmental Toxicity: Rat	69	39	Marginal
30	Developmental Toxicity: Rabbit	0	0	I
31	Developmental Toxicity: Human	0	0	I
32	Cellular Toxicity [3T3]	0	0	I(IC ₅₀ >1 μ M)
33	Cellular Toxicity [HeLa]	0	0	I(IC ₅₀ >7mM)
34	Rat Lethality [LD ₅₀]	0	0	I(>7.2 mmol/kg)

I = Inactive

A = Active

* See text for discussion

Additionally, the vast majority of *genotoxic* rodent carcinogens induce cancers at multiple sites of multiple species [47]. On the other hand, GBP, like most non-genotoxic rodent carcinogens, induces cancers at a single site of a single gender of one species [59].

Using a previously described battery of SAR models of rodent carcinogenicity [49,50], GBP was not predicted to induce cancers in rat (Table 1, Analyses Nos. 18 and 21)(the species previously reported to elicit pancreatic acinar tumors in response to GBP [59]) but to do so in mice, based upon one (CPDB) of the two SAR rat carcinogenicity models (Table 1, Analysis No. 22). The other projections indicated lack of carcinogenicity (Table 1, Analyses Nos. 17–21). Based upon the integration of all these rodent carcinogenicity predictions, GBP is projected to have a “marginal” probability (0.533) of non-genotoxic carcinogenicity [50]. (In that paradigm, “carcinogenicity” is defined as a probability of ≥ 0.6).

However, further examination of the basis of the prediction of carcinogenicity of GBP in mice indicates (a) that it is based upon a putative structural determinant that is associated with a single mouse carcinogen in the database, this implies a low confidence level (*i.e.* 50%), and (b) the putative toxicophore is derived from a mouse carcinogen (calciferol) in the database that is structurally dissimilar from GBP (compare Figures 1A and 1B). Based upon these considerations, we felt justified in downgrading the prediction to mouse non-carcinogen. Accordingly, assuming a negative prediction of mouse carcinogenicity, together with the other negative carcinogenicity predictions (Table 1, Analyses Nos. 17–21), the overall probability that GBP is a rodent carcinogen decreases to 0.154 [50], *i.e.* GBP is predicted, with a high degree of certainty, to be both non-genotoxic and non-carcinogenic.

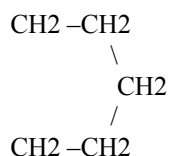
The neoplastic transformation of cultured cells by chemicals is taken as an indication of a potential for carcinogenicity [52]. GBP lacks that potential (Table 1, Analysis No. 13). Binding to the *Ah* receptor may initiate a cascade of events that interfere with cell signaling and apoptosis [69]. This can, ultimately, result in carcinogenesis. GBP does not exhibit a potential to bind to the *Ah* receptor (Table 1, Analysis No. 24). These findings further support the conclusion that GBP does not represent an unacceptable risk to humans.

One of the mechanisms for non-genotoxic carcinogenicity involves cell or systemic toxicity resulting in mitogenesis and ultimately tumor development [70–74]. GBP, however, lacks the potential to induce toxicity in cultured human HeLa (dye exclusion assay) or cultured murine Balb3T3 clonal assay) cells (Table 1, Analyses Nos. 32 and 33). Additionally the acute toxicity of GBP in rats (LD₅₀) is predicted to be low (> 7.2 mmol/kg; Table 1, Analysis No. 34). In fact, the experimentally determined LD₅₀ value is reported to be in excess of 46.8 mmol/kg [66,67].

The MTD (maximum tolerated dose) values of GBP in mice and rats are also predicted to be high, *i.e.* low systemic toxicity (> 0.9 and > 1.8 mmol/kg, respectively) (Table 1, Analyses Nos. 16 and 15). This is supported by the experimentally determined MTD values in rats and mice which

are approximately 12 mmol/kg [59,67]. In fact, the pancreatic acinar tumors in male rats occurred in the range of these high MTD values [59]. This might be part of the mechanistic basis of the observed carcinogenicity in male rats. Additionally, while GBP was not found to induce mitogenesis in male rat pancreatic acinar cells, it did so in cultured normal pancreatic acinar cells albeit the level of mitogenesis was low [65]. Obviously, even if these findings were applicable to humans, it is doubtful that humans will tolerate and be maintained on such high doses of GBP for prolonged periods of time.

The molecule contains the Toxicophore (nr. occ. = 1):



*** 3 out of the known 4 molecules (75%) containing such a Toxicophore are iGJIC

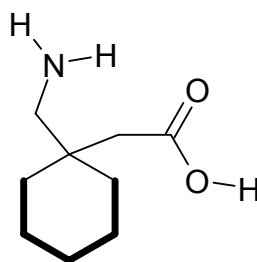


Figure 2. Prediction of the potential of GBP to inhibit gap junctional intercellular communication (GJIC). The potential is due to the toxicophore shown in bold. The toxicophore is derived from 4 chemicals in the data base. Three of these are active inhibitors of GJIC and one is marginally active. The structure of the four chemicals is shown in Figure 3.

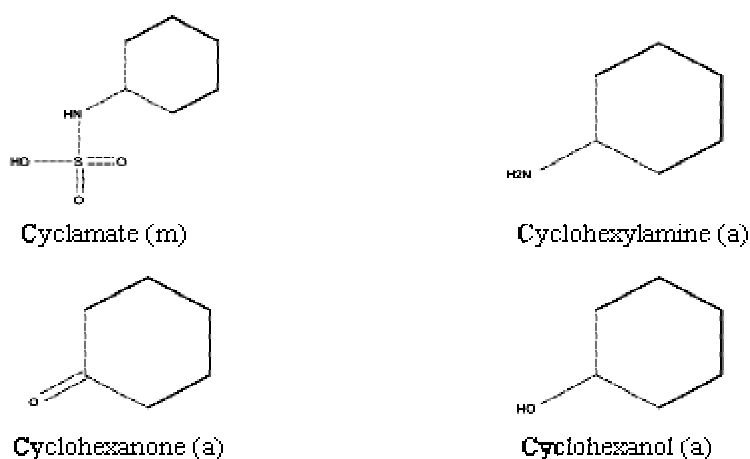


Figure 3. Structures of the chemicals in the data base that are responsible for the toxicophore (Figure 2) associated with the potential of GBP to inhibit gap junctional intercellular communication (GJIC). “a” and “m” indicate activity and marginal activity, respectively.

Inhibition of gap junctional intercellular communication (GJIC) is taken as an indication of a potential for tumor promotion by an epigenetic (non-genotoxic mechanism) [75]. GBP was found to exhibit such a potential (Figure 2, Table 1, Analysis No. 12). Moreover, the chemicals in the database that generated the toxicophore responsible for the prediction had structures relevant to that of GBP (Figure 3). Thus, inhibition of GJIC might provide a mechanism for the carcinogenic progression of the pancreatic acinar cells in male rats after they are stimulated by mitogenesis.

The molecule contains the Toxicophore (nr. occ. = 1):

CO –CH₂

*** 6 out of the known 6 molecules (100%) containing such a Toxicophore
are inducers of α 2 μ -Nephropathy (conf. level = 98%)

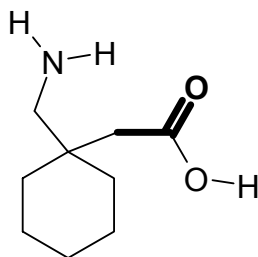


Figure 4. Prediction of the potential of GBP to induce α 2 μ -globulin associated nephropathy. The potential derives from the toxicophore (shown in bold) that is present in the 6 active chemicals shown in Figure 5.

The induction of α 2 μ -globulin-associated nephropathy [76] is taken as a mechanism for the induction of renal tumors in male rats. (This phenomenon is *not* associated with a carcinogenic risk for humans.) GBP is predicted to have such a potential (Figure 4, Table 1, Analysis No. 26). This confirms the experimental finding that GBP induces that nephropathy [77].

However, GBP, unlike most other inducers of α 2 μ -globulin-nephropathy, was found experimentally *not* to induce the α 2 μ -nephropathy related tumors in male rats [59]. An examination of the structural basis of the prediction of α 2 μ -associated nephropathy suggests a dichotomy. Thus most of the chemicals that contribute to the relevant structural determinant have the toxicophore embedded in carbonyl-containing moiety (Figure 5), while in GBP, it is within a carboxyl group (Figure 4). The eventual elucidation of this observation will require the development of an SAR model based upon experimental results of a much larger group of chemicals. However, it may be that when embedded in a carboxyl moiety the toxicophore induces a nephropathy that does not result in the renal tumor. In fact, recently other chemicals capable of inducing the nephropathy but not the renal tumors, have also been identified [78].

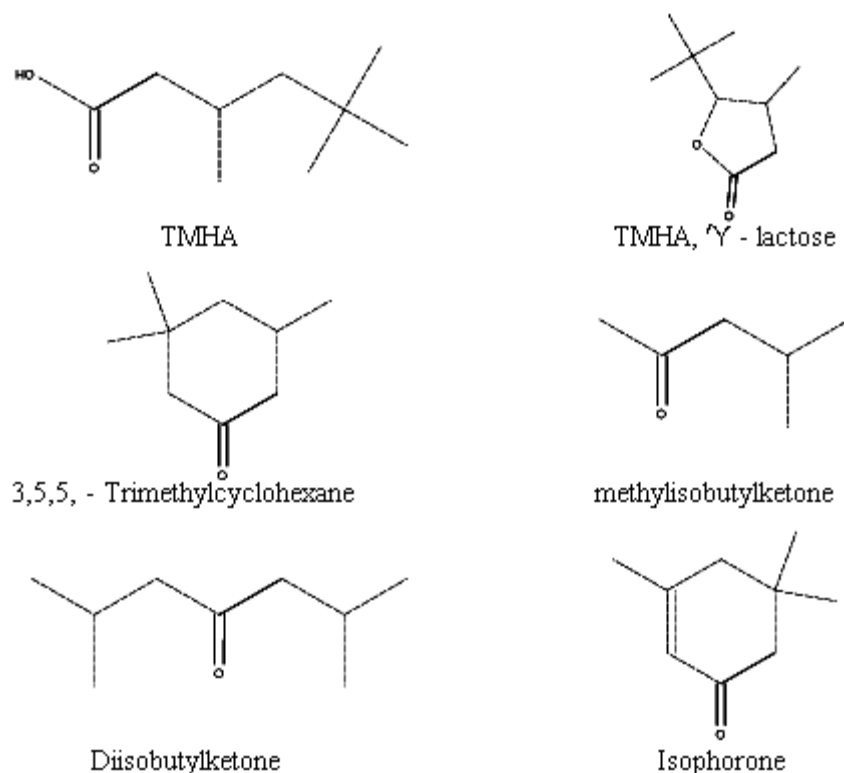


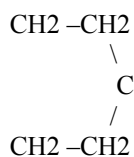
Figure 5. Structures of the chemicals in the data base that are responsible for the toxicophore associated with the potential of GBP to induce $\alpha_2\mu$ -globulin associated nephropathy. Abbreviation: TMHA = 3,5,5-Trimethyl hexanoic acid.

Inhibition of the human cyp2D6 is not a toxic manifestation *per se*. Indeed it is a property shared by toxicants (e.g. 1, 2, 3, 6-tetrahydro-1-methyl-4-phenylpyridine (MPTP)) as well as medicinals (e.g. nocardipine, budipine, chlorpromazine) [54,79,80]. However, blockage of that enzyme by GBP may interfere with the detoxification of GBP or of a co-administered agent (*i.e.* drug-drug interaction) [81].

While it is assumed that GBP is not metabolized further [15,22], the finding of GBP's potential for inhibiting cyp2D6 (Figure 6, Table 1, Analysis No. 25) may provide a mechanism for the toxicity that is observed when GBP is co-administered together with other antiepileptic agents (AEDs) (e.g. carbamazepine, amitriptyline or phenobarbital) [19,82–84]. These AEDs do not block cyp2D6 [54] but their detoxification might be blocked by a GBP-induced inhibition of that isozyme.

Perturbation of tubulin polymerization is a phenomenon that can lead to cell toxicity as well as the induction of micronuclei by a mechanism involving aneuploidy [85]. As GBP is not predicted to induce micronuclei *in vivo* (Table 1, Analysis No. 7), malsegregation (*i.e.* aneuploidy) (Table 1, Analysis No. 11) or cell toxicity (Table 1, Analyses Nos. 32 and 33), it is not unexpected that GBP also does not show a potential for perturbing tubulin polymerization (Table 1, Analysis No. 14).

The molecule contains the Biophore (nr.occ. = 1)



*** 8 out of the known 8 molecules (100%) containing such a Biophore are inhibitors of cyp2D6

*** QSAR Contribution: Constant is: 72.15

** The following Modulators are also present:

Log partition coeff. = 0.54; LogP**2 contribution is 0.22
Hard/Soft index is = 1.14; Its contribution is -45.05

** Total projected QSAR activity 27.32

** The projected cyp2D6 inhibiting potency is 27.3 SAR units **

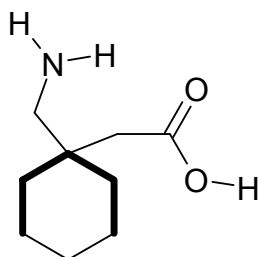


Figure 6. Prediction of the ability of GBP to inhibit human cyp2D6. That potential is associated with the biophores shown in bold. A projected potency of 27.3 SAR units indicates moderately weak inhibition.

The molecule contains the expanded Toxicophore (nr.occ. = 1):

(A) NH₂–CH₂

*** 1 out of the known 1 molecules containing such a Toxicophore is a developmental toxicant (conf.level= 50%)

*** This biophore is not statistically significant ***

The molecule also contains the expanded Toxicophore:

(B) CH₂–CH₂–CH₂–C–

*** 1 out of the known 1 molecules (100%) containing such a Biophore is a developmental toxicant (conf.level= 50%)

*** This biophore is not statistically significant ***

*** The probability that this molecule is a Developmental Toxicant is increased to 69% due to the potency of the extra Toxicophore

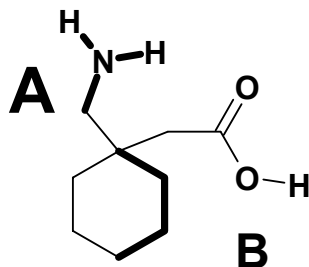


Figure 7. Prediction of the questionable potential of GBP to induce a developmental effect in rats. That suspicion derives from the two putative toxicophores (A and B) shown in bold. However, each is present in only one chemical in the database. Note that GBP is *not* predicted to induce developmental effects either in humans or in other species (see Table 1).

GBP was predicted not to induce developmental effects in mice, hamsters and rabbits (Table 1, Analyses Nos. 27, 28 and 30). The lack of effects in mice and rabbits has been described previously [66,86]. GBP exhibited a slight potential for inducing developmental effects in rats (Figure 7, Table 1, Analysis No. 29).

However, that potential is based on two putative toxicophores, each present in only one chemical in the database and, thus, it is at best an alert for a possible effect. It is interesting, however, that it was found that GBP induced a slight but significant increased incidence of dilated renal pelvis in rats. However, this observation was not considered biologically significant [66]. Thus, the suspicious SAR prediction for developmental effects in rats (Table 1, Analysis No. 29, Figure 7) parallels the marginal experimental findings.

Significantly, however, using two separate SAR models of developmental effects in humans, GBP was predicted to lack such a potential (Table 1, Analysis No. 31). It has also been reported that 25–30% of early development effects are due to mutational and/or chromosomal events [87–89]. As GBP is devoid of these potentials (Table 1, Analyses Nos. 1–11), this further supports the absence of a potential for developmental toxicity.

4 CONCLUSIONS

The available experimental data on the toxicity of GBP were confirmed by the SAR models. Moreover, in examining the potential for toxicity of GBP, the SAR analyses, based both on genotoxicity and ancillary assays, indicate a low probability that it poses a carcinogenic hazard to humans. The analyses suggest that the reported carcinogenicity of GBP in male rats (but not in female rats and not in mice) reflects a non-genotoxic potential seen at high dosages, at or near the MTD, that results in systemic toxicity, mitogenesis and tumor promotion.

While GBP has a slight potential for inducing developmental effects in rats, the analyses do not indicate a potential for inducing developmental effects in humans either by a stage-specific or a mutagenic/chromosomal mechanism.

It must be stressed that the projections presented herein are hazard identifications. Obviously, the realization of the potential, if any, depends upon the dose, co-exposure to other agents, homeostatic and genetically-determined defense and repair mechanisms. Altogether, the low probability of untoward side effects together with the proven therapeutic efficacy of GBP are very favorable outcomes of this analysis.

Finally the present study indicates that (a) SAR models predict independently obtained experimental results and (b) the application of these computational methods to hazard identification of therapeutics, while promising, must be done in the context of human expertise and intuition.

Acknowledgment

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5 REFERENCES

- [1] I. D. McKinney, A. Richard, C. Waller, M. C. Newman and F. Gerberick, The practice of structure–activity relationships (SAR) in toxicology, *Toxicol. Sci.* **2000**, 56, 8–17.
- [2] CEC, Commission of the European Communities. White Paper: Strategy for a Future Chemicals Policy, **2001**, <http://europa.eu.int/comm/environment/chemicals/whitepaper.htm>.
- [3] E. M. Hulzebos and R. Posthumus, (Q)SARS: Gatekeepers against risk on chemicals?, *SAR QSAR Environ. Res.* **2003**, 14, 285–316.
- [4] M. T. D. Cronin, J. S. Jaworska, J. D. Walker, M. H. I. Comber, C. D. Watts and A. P. Worth, Use of QSARs in international decision–making frameworks to predict health effects of chemical substances, *Environmental Health Perspectives* **2003**, 111, 1391–1401.
- [5] ECOTOC, Workshop on regulatory acceptance of (Q)SARS for human health and environmental endpoints. European Centre for Ecotoxicology and Toxicology of Chemicals. March 4–6, 2002. www.ecetoc.org. *Setubal, Portugal* **2002**.
- [6] J. D. Walker, L. Carlsen and J. Jaworska, Improving opportunities for regulatory acceptance of QSARS: The importance of model domain, uncertainty, validity and predictability, *Quantitative Structure–Activity Relationships* **2003**, 22, 346–350.
- [7] J. S. Jaworska, M. Comber, C. Auer and C. J. Van Leeuwen, Summary of a workshop on regulatory acceptance of (Q)SARs for human health and environmental endpoints, *Environmental Health Perspectives* **2003**, 111, 1358–1360.
- [8] L. Eriksson, J. Jaworska, A. P. Worth, M. T. D. Cronin, R. M. McDowell and P. Gramatica, Methods for reliability, uncertainty assessment, and for applicability evaluations of classification and regression based QSARs, *Environmental Health Perspectives* **2003**, 111, 1361–1375.
- [9] H. S. Rosenkranz, A. R. Cunningham, Y. P. Zhang, H. G. Claycamp, O. T. Macina, N. B. Sussman, S. G. Grant and G. Klopman, Development, characterization and application of predictive–toxicology models, *SAR QSAR Environ. Res.* **1999**, 10, 277–298.
- [10] H. S. Rosenkranz, SAR in the Assessment of Carcinogenesis: The MULTICASE approach; in: *Quantitative Structure–Activity Relationship (QSAR) Models of Mutagens and Carcinogens*, Eds. R. Benigni, CRC Press, LLC, Boca Raton, FL, 2003, pp. 175–206.
- [11] H. S. Rosenkranz, Structural concepts in the prediction of the toxicity of therapeutical agents; in: *Burger's Medicinal Chemistry and Drug Discovery*, Eds. John Wiley & Sons, New York, 2003, pp. 827–847.
- [12] W. J. Curry and D. L. Kulling, Newer antiepileptic drugs: Gabapentin, Lamotrigine, Felbamate, Topiramate and Fosphenytoin, *American Family Physician* **1998**, February 1, 1998, 1–11.
- [13] D. Chadwick, Gabapentin, *Lancet* **1994**, 343, (January 8, 1994), 89–91.
- [14] M. Gasior, J. T. Ungard and J. M. Witkin, Preclinical evaluation of newly approved and potential antiepileptic drugs against cocaine–induced seizures, *The Journal of Pharmacology and Experimental Therapeutics* **1999**, 290, 1148–1156.
- [15] Studies and Information – Gabapentin – RxList Monographs, **2003**, http://www.rxlist.com/cgi/generic/gabapent_cp.htm.
- [16] NIH, Gabapentin (Systemic), **2003**, <http://www.nlm.nih.gov/medlineplus/druginfo/uspdi/202732.html>.
- [17] L. V. Wilton and S. Shakir, A postmarketing surveillance study of Gabapentin as add–on therapy for 3,100 patients in England, *Epilepsia* **2002**, 43, (9), 983.
- [18] W. Klein–Schwartz, J. G. Shepherd, S. Gorman and B. Dahl, Characterization of gabapentin overdose using a poison center case series, *J. Toxicol Clin Toxicol.* **2003**, 41, 11–15.
- [19] V. D. Hsu, S. Alemyehy and E. Barry, Gabapentin induced hepatotoxicity. *ASHP (American Society of Health Systems Pharmacists) Annual Meeting* **1995**, June 13, 52.
- [20] J. H. Fischer, A. N. Barr, S. L. Rogers, P. A. Fischer and V. L. Trudeau, Lack of serious toxicity following gabapentin overdose, *Neurology* **1994**, 44, 982–983.
- [21] M. J. McLean, Gabapentin, *Epilepsia* **1995**, 36 Suppl 2, S73–86.
- [22] K. O. Vollmer, A. von Hodenberg and E. U. Kolle, Pharmacokinetics and metabolism of gabapentin in rat, dog, and human, *Arzneimittel–Forschung* **1986**, 36, 830–839.
- [23] G. Klopman and H. S. Rosenkranz, Prediction of carcinogenicity/mutagenicity using MULTICASE, *Mutation Research* **1994**, 305, 33–46.
- [24] G. Klopman and H. S. Rosenkranz, Toxicity estimation by chemical substructure analysis: The Tox II Program,

- Toxicology Letters* **1995**, *79*, 145–155.
- [25] L. Eriksson, E. Johansson and S. Wold, QSAR model validation; in: *Quantitative structure–activity relationships in environmental sciences. Proceedings of the 7th International Workshop on QSAR in Environmental Sciences, June 24–28, Elsinore, Denmark*, Eds. F. Chen and G. Schüürmann, SETAC Press, Pensacola, FL, 1997, pp. 381–397.
- [26] A. Tropsha, P. Gramatica and V. K. Gombar, The importance of being earnest: Validation is the absolute essential for successful application and interpretation of QSPR Models, *Quant. Struct. Activ. Rel.* **2003**, *22*, 69–77.
- [27] Y. P. Zhang, N. Sussman, G. Klopman and H. S. Rosenkranz, Development of methods to ascertain the predictivity and consistency of SAR models: application to the US National Toxicology Program rodent carcinogenicity bioassays, *Quantitative Structure–Activity Relationships* **1997**, *16*, 290–295.
- [28] H. S. Rosenkranz, A data mining approach for the elucidation of the action of putative etiological agents: Application to the non–genotoxic carcinogenicity of genistein, *Mutation Res.* **2003**, *526*, 85–92.
- [29] H. S. Rosenkranz, Synergy between systemic toxicity and genotoxicity: Relevance to human cancer risk, *Mutation Research* **2003**, *529*, 117–127.
- [30] M. Rosenkranz, H. S. Rosenkranz and G. Klopman, Intercellular communication, tumor promotion and non–genotoxic carcinogenesis: relationships based upon structural considerations, *Mutation Research* **1997**, *381*, (2), 171–188.
- [31] H. S. Rosenkranz and G. Klopman, Structural alerts to genotoxicity: The interaction of human and artificial intelligence, *Mutagenesis* **1990**, *5*, 333–361.
- [32] J. Ashby, Fundamental structural alerts to potential carcinogenicity or non–carcinogenicity., *Environm. Mutag.* **1985**, *7*, 919–921.
- [33] B. Henry, S. G. Grant, G. Klopman and H. S. Rosenkranz, Induction of forward mutations at the thymidine kinase locus of mouse lymphoma cells: Evidence for electrophilic and non–electrophilic mechanisms, *Mutation Res.* **1998**, *397*, 313–335.
- [34] H. S. Rosenkranz, F. K. Ennever, M. Dimayuga and G. Klopman, Significant differences in the structural basis of the induction of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells, *Environ. Mol. Mutagen.* **1990**, *16*, 149–177.
- [35] W.–L. Yang, G. Klopman and H. S. Rosenkranz, Structural basis of the *in vivo* induction of micronuclei, *Mutation Res.* **1992**, *272*, 111–124.
- [36] A. Labbauf, G. Klopman and H. S. Rosenkranz, Dichotomous relationship between DNA reactivity and the induction of sister chromatid exchanges *in vivo* and *in vitro*, *Mutation Res.* **1997**, *377*, 37–52.
- [37] M. Liu, S. G. Grant, O. T. Macina, G. Klopman and H. S. Rosenkranz, Structural and mechanistic bases for the induction of mitotic chromosomal loss and duplication ('malsegregation') in the yeast *Saccharomyces cerevisiae*: Relevance to human carcinogenesis and developmental toxicology, *Mutation Research* **1997**, *374*, 209–231.
- [38] E. ter Haar, H. S. Rosenkranz, E. Hamel and B. W. Day, Computational and molecular modeling evaluation of the structural basis for tubulin polymerization inhibition by colchicine site agents., *Bioorganic Med. Chem.* **1996**, *4*, 1659–1671.
- [39] U. Rannug, M. Sjogren, A. Rannug, M. Gillner, R. Toftgard, J. A. Gustafsson, H. Rosenkranz and G. Klopman, Use of artificial intelligence in structure–affinity correlations of 2,3,7,8–tetrachlorodibenzo–*P*–dioxin (TCDD) receptor ligands, *Carcinogenesis* **1991**, *12*, 2007–2016.
- [40] N. Takihi, H. S. Rosenkranz, G. Klopman and D. R. Mattison, Structural determinants of developmental toxicity, *Risk Analysis* **1994**, *14*, 649–657.
- [41] J. Gomez, O. T. Macina, D. R. Mattison, Y. P. Zhang, G. Klopman and H. R. Rosenkranz, Structural determinants of developmental toxicity in hamsters., *Teratology* **1999**, *60*, 190–205.
- [42] M. Ghanooni, Y. P. Mattison, Y. P. Zhang, O. T. Macina, H. S. Rosenkranz and G. Klopman, Structural determinants associated with risk of human developmental toxicity, *Amer. J. Obstetrics Gynecology* **1997**, *76*, 799–806.
- [43] M. Liu, N. Sussman, G. Klopman and H. S. Rosenkranz, Estimation of the optimal data base size for structure–activity analyses: The Salmonella mutagenicity data base, *Mutation Research* **1996**, *358*, 63–72.
- [44] E. Zeiger, J. Ashby, G. Bakale, K. Enslein, G. Klopman, and H. S. Rosenkranz, Prediction of *Salmonella* mutagenicity, *Mutagenesis* **1996**, *11*, 471–484.
- [45] V. Mersch–Sundermann, U. Schneider, G. Klopman and H. S. Rosenkranz, SOS–Induction in *E. coli* and *Salmonella* mutagenicity: A comparison using 330 compounds, *Mutagenesis* **1994**, *9*, 205–224.
- [46] V. Mersch–Sundermann, G. Klopman and H. S. Rosenkranz, Chemical structure and genotoxicity: Studies of the SOS Chromotest, *Mutation Research* **1996**, *340*, 81–91.
- [47] J. Ashby and R. W. Tennant, Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP, *Mutation Research* **1991**, *257*, 229–306.
- [48] L. S. Gold, T. H. Slone and B. N. Ames, Overview and update of analyses of the carcinogenic potency database; in: *Handbook of Carcinogenic Potency and Genotoxicity Databases*, Eds. L. S. Gold and E. Zeiger, CRC Press,

- Boca Raton, FL, 1997, pp. 661–685.
- [49] Y. P. Zhang, N. Sussman, O. T. Macina, H. S. Rosenkranz and G. Klopman, Prediction of the carcinogenicity of a second group of chemicals undergoing carcinogenicity testing, *Environmental Health Perspectives* **1996**, *104*, (Suppl. 5), 1045–1050.
- [50] O. T. Macina, Y. P. Zhang and H. S. Rosenkranz, Improved predictivity of carcinogens: the use of a battery of SAR models; in: *Testing, Predicting and Interpreting Carcinogenicity*, Eds. K. T. Kitchen, Marcel Dekker, New York, NY, 1998, pp. 227–250.
- [51] Y. P. Zhang, A. vanPraag, G. Klopman and H. S. Rosenkranz, Structural basis of the induction of unscheduled DNA synthesis in rat hepatocytes, *Mutagenesis* **1994**, *9*, 141–149.
- [52] E. J. Matthews, J. W. Spalding and R. W. Tennant, Transformation of BALB/C–3T3 cells. V. Transformation responses of 166 chemicals compared with mutagenicity in Salmonella and carcinogenicity in rodent bioassay, *Environmental Health Perspectives* **1993**, *101*, 347–482.
- [53] X. Zhu and H. S. Rosenkranz, Structural basis of the toxicity of chemicals in cultured human HeLa cells, *ATLA* **2000**, *28*, 557–574.
- [54] G. R. Strobl, S. von Kruedener, J. Stockigt, F. P. Guengerich and T. Wolff, Development of a pharmacophore for inhibition of human liver cytochrome P–450 2D6: molecular modeling and inhibition studies., *J. Med. Chem.* **1993**, *36*, 1136–1145.
- [55] G. Reifferscheid and J. Heil, Validation of the SOS/*umu* test using results of 486 chemicals and comparison with the Ames test and carcinogenicity data., *Mutation Res.* **1996**, *369*, 129–145.
- [56] NIOSH, Registry of Toxic Effects of Chemical Substances (RTECS), **2003**, <http://www.cdc.gov/niosh/rtecs.html>.
- [57] H. S. Rosenkranz and G. Klopman, Structural relationships between mutagenicity, maximum tolerated dose, and carcinogenicity in rodents, *Environmental and Molecular Mutagenesis* **1993**, *21*, 193–206.
- [58] Indications, Dosage and Administration – Gabapentin – RXList Monographs, **2003**, http://www.rxlist.com/cgi/generic/gabapent_ids.htm.
- [59] R. E. Sigler, A. W. Gough and F. A. de la Inglesia, Pancreatic acinar cell neoplasia in male Wistar rats following 2 years of gabapentin exposure, *Toxicology* **1995**, *98*, 73–82.
- [60] F. K. Ennever, T. J. Noonan and H. S. Rosenkranz, The predictivity of animal bioassays and short–term genotoxicity tests for carcinogenicity and non–carcinogenicity to humans, *Mutagenesis* **1987**, *2*, 73–78.
- [61] H. Bartsch and C. Malaveille, Prevalence of genotoxic chemicals among animal and human carcinogens evaluated in the IARC Monograph Series, *Cell Biol. and Toxic.* **1989**, *5*, 115–127.
- [62] M. D. Shelby, The genetic toxicity of human carcinogens and its implications, *Mutation Res.* **1988**, *204*, 3–15.
- [63] J. Ashby and R. S. Morrod, Detection of human carcinogens, *Nature* **1991**, *352*, 185–186.
- [64] G. W. Lucier, Receptor–mediated carcinogenesis.; in: *Mechanisms of Carcinogenesis in Risk Identification*, Eds. P. N. M. H. Vainio, D.B. McGregor and O.J.McMichael, International Agency for Research on Cancer, Lyon, 1992, pp. 87–112.
- [65] L. Dethloff, B. Barr, L. Bestervelt, S. Bulera, R. E. Sigler, M. LaGattuta and F. de la Iglesia, Gabapentin–induced mitogenic activity in rat pancreatic acinar cells, *Toxicological Sciences* **2000**, *55*, 52–59.
- [66] J. A. Petrere and J. A. Anderson, Developmental toxicity studies in mice, rats, and rabbits with the anticonvulsant gabapentin, *Fundamental and Applied Toxicology* **1994**, *23*, 585–589.
- [67] G. D. Bartoszyk, N. Meyerson, W. Reimann, G. Satzinger and A. von Hodenberg, Gabapentin; in: *New Anticonvulsant Drug*, Eds. B. S. Meldrum and R. J. Porter, John Libby, London, 1986, pp. 147–163.
- [68] V. Chankong, Y. Y. Haimes, H. S. Rosenkranz and J. Pet–Edwards, The carcinogenicity prediction and battery selection (CPBS) method: A Bayesian approach, *Mutation Res.* **1985**, *153*, 135–166.
- [69] G. Koss and D. Wölfle, Dioxin and dioxin–like polychlorinated hydrocarbons and biphenyls; in: *Toxicology*, Eds. H. Marquardt, S. G. Schäfer, R. McClellan and F. Welsch, Academic Press, San Diego, 1999, pp. 699–728.
- [70] B. N. Ames and L. S. Gold, Too many rodent carcinogens: Mitogenesis increases mutagenesis, *Science* **1990**, *249*, 970–971.
- [71] S. Preston–Martin, M. C. Pike, R. K. Ross, P. A. Jones and B. E. Henderson, Increased cell division as a cause of human cancer, *Cancer Res.* **1990**, *50*, 7415–7421.
- [72] B. E. Butterworth, Consideration of both genotoxic and nongenotoxic mechanisms in predicting carcinogenic potential, *Mutation Res.* **1990**, *239*, 117–132.
- [73] S. M. Cohen and L. B. Ellwein, Cell proliferation in carcinogenesis, *Science* **1990**, *249*, 1007–1011.
- [74] S. M. Cohen and L. B. Ellwein, Genetic errors, cell proliferation and carcinogenesis, *Cancer Res.* **1991**, *51*, 6493–6505.
- [75] J. E. Trosko, C. C. Chang, B. Upham and M. Wilson, Epigenetic toxicology as toxicant–induced changes in intracellular signaling leading to altered gap junctional intercellular communication, *Toxicol. Lett.* **1998**, *102–103*, 71–78.
- [76] J. A. Swenberg, B. Short, S. Borghoff, J. Strasser and M. Charbonneau, The comparative pathobiology of α 2u – globulin nephropathy, *Toxicol. Appl. Pharmacol.* **1989**, *97*, 35–46.

- [77] M. A. Dominick, D. G. Robertson, M. R. Bleavins, R. E. Sigler, W. F. Bobrowski and A. W. Gough, Alpha 2 μ -globulin nephropathy without nephrocarcinogenesis in male Wistar rats administered 1-(aminomethyl) cyclohexaneacetic acid, *Toxicol. Appl. Pharmacol.* **1991**, *111*, 375–387.
- [78] H. Marquardt, Chemical carcinogenesis; in: *Toxicology*, Eds. H. Marquardt, S. G. Schafer, R. McClellan and F. Welsch, Academic Press, San Diego, 1999, pp. 151–178.
- [79] M. de Groot, M. J. Ackland, V. A. Horne, A. A. Alex and B. C. Jones, A novel approach to predicting P450 mediated drug metabolism. CYP2D6 catalyzed N-dealkylation reactions and qualitative metabolite predictions using a combined protein and pharmacophore model for CYP2D6, *J. Med. Chem.* **1999**, *42*, 4062–4070.
- [80] M. de Groot, M. J. Ackland, V. A. Horne, A. A. Alex and B. C. Jones, Novel approach to predicting P450-mediated drug metabolism: Development of a combined protein and pharmacophore model for CYP2D6, *J. Med. Chem.* **1999**, *42*, 1515–1524.
- [81] H. Rosenkranz, Computational toxicology and the generation of mechanistic hypotheses: Gamma-butyrolactone, *SAR QSAR Environ. Res.* **2001**, *12*, 435–444.
- [82] C. E. Heughan and J. Sawynok, The interaction between gabapentin and amitriptyline in the rat formalin test after systemic administration, *Anesth. Analg.* **2002**, *94*, 975–980.
- [83] A. Sanchez-Romero, J. A. Duran-Quintana, R. Garcia-Delgado, C. Margarito-Rangel and J. L. Poveda-Andres, Possible gabapentin phenytoin interaction, *Rev Neurol* **2002**, *16*, 952–953.
- [84] Neurontin side effects, interactions, reactions, pediatric, geriatric – Gabapentin, **2003**, http://www.rxlist.com/cgi/generic/gabapent_ad.htm.
- [85] E. ter Haar, B. W. Day and H. S. Rosenkranz, Direct tubulin polymerization perturbation contributes significantly to the induction of micronuclei in vivo., *Mutation Res.* **1996**, *350*, 331–337.
- [86] A. Sabers, Teratogenic effects of old and new antiepileptic drugs, *Epilepsia* **1998**, *39* (Suppl 2), 45.
- [87] E. Hodgson and P. E. Levi, *A Textbook of Modern Toxicology*. Elseviers, Amsterdam, **1979**.
- [88] J. B. Bishop, K. L. Witt and R. A. Sloane, Genetic toxicities of human teratogens, *Mutation Research* **1997**, *396*, 9–43.
- [89] J. M. Rogers and R. J. Kavlock, Developmental toxicology; in: *Casarett and Doull's Toxicology. The Basic Science of Poisons. Fifth Edition*, Eds. C. D. Klaassen, McGraw-Hill, New York, 1996, pp. 301–331.