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Ecotoxicological Quantitative Structure–Activity Relationships for Pharmaceuticals

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Abstract This paper examined active pharmaceutical ingredients (APIs) acute ecotoxicological modes of action (MOA). It was concluded that the vast majority of APIs acute MOA was non-specific narcosis as; 85% out of 59 APIs had an excess toxicity ratio <7; 70% of the APIs ecotoxicity was overestimated based on a narcotic model; and the majority of APIs Log EC_{50}-Log K_{ow} regression slopes (−0.49 to −0.86) were within the range of the universal narcosis slopes. However, hydrophobicity is likely not the proper descriptor for assessment of pharmacodynamic APIs chronic ecotoxicity, to asses this accurately new experimental methods need development.

Keywords Pharmaceuticals · Ecotoxicological QSAR · Modes-of-action · Narcosis

Active pharmaceutical ingredients (APIs) have been reported ubiquitously in surface waters in the low µg/L range (Kolpin et al. 2002). This has prompted legitimate public concerns (Daughton and Jones-Lepp 2001), as APIs are biologically active compounds with use rates and environmental release amounts comparable to agricultural chemical (Jones et al. 2001). Currently there are ecotoxicological data available for only a few percent of the APIs on the market in the open literature according to the United States National Oceanic and Atmospheric Administration (NOAA 2006), hence quantitative structure–activity relationship [(Q)SAR] generated data is the first step needed in gaining a more general knowledge on the issue of APIs in the environment (Jørgensen and Halling-Sørensen 2000), hence the European Union Commission's Scientific Committee on Toxicity, Ecotoxicity and Environment (CSTEE) have recommended use of (Q)SAR models for screening of APIs ecotoxicity (EU 2001). (Q)SARs have previously been shown to generate conservative estimates of APIs acute (24 h to 21 days) aquatic toxicity relative to measured data based on standardized tests (Sanderson et al. 2003). However, the model's accuracy relative to APIs known or expected specific modes-of-action (MOA) (Seiler 2002), which in part is triggering and/or adding to public and regulatory environmental concerns (Daughton and Jones-Lepp 2001) has only been assessed in greater detail for a few APIs. For these few APIs it was concluded that the primary MOA is narcosis (Escher et al. 2002, 2005).

The toxic MOA narcosis is a reversible non-specific state of arrested activity of proplasmic structures (Veith and Broderius 1990), also referred to as baseline toxicity and is defined as the minimal toxicity that a compound may elicit (Escher et al. 2002). The important aspect in relation to APIs to note is the non-specific MOA that narcosis represent, and the fact that APIs based on their bioactivity (Seiler 2002) would be expected not to act primarily via narcosis.

This paper will demonstrate both adapted (Q)SARs specific for APIs based on existing and publicly available models used by the US Environment Protection Agency (USEPA), and new empirical models for APIs in the tradition of e.g. Hansch et al. (1995). The aim is to address the question whether APIs pose a special/novel potential hazard to the aquatic environment by broadening the discussion on APIs' acute toxic MOA to cover more APIs in a weight-of-evidence approach. Can Escher et al. (2002, 2005) conclusions regarding narcosis be generalized to cover more APIs – or is Seiler (2002) concerns of specific

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MOAs characteristic of APIs due to their pharmacodynamic design and shared receptor systems between target and non-target organisms more accurate?

Materials and Methods

This assessment utilizes multiple linear regressions of the Log(EC_{50})–Log(K_{ow}) relationship for fish, daphnids and algae to derive distinct slopes of the regressions which then are compared to the range of the universal narcosis slopes (McGrath et al. 2004) ranging from −0.65 to −0.95 (Russom et al. 1997). This will yield a first screening level indication of pharmaceuticals' general ecotoxicological MOA, with the thesis that the generated regressions slopes must be outside the range of the universal narcosis slope range in order for APIs toxicity to be driven by specific MOAs and not primarily by narcosis.

There are two principal data sources for this study. The first is the database generated by Sanderson et al. (2004) covering all 50 different pharmaceutical classes identified in the Martindale (2002) complete drug reference from analgesics, over hormones to cancer drugs, and 2,575 APIs based on (Q)SAR predictions from the USEPA model ECOSAR v3.20 (USEPA 2007). ECOSAR is based on approximately 150 (Q)SARs for 50 different compound classes where toxicity is predicted with Log K_{ow} as the prime descriptor (Sanderson et al. 2004). The second source of data is the NOAA (2006) database of measured acute aquatic ecotoxicity values. This database was chosen because of the transparency, comprehensiveness, and data quality control on entered data performed by NOAA. The database covers nine different classes of APIs, with an emphasis on highly prescribed APIs, in this analysis 59 APIs with daphnid (n = 71) and fish (n = 73) data were utilized, a few APIs had more than one study and toxicity value available. The NOAA (2006) database does not include algae data thus precluding assessment of APIs MOA based on measured data towards algae. As always with compiled databases from various sources inter-laboratory variability introduces noise in respect to precision of the regression. Moreover, five different fish species were used in the toxicity testing thus contributing noise to the inter-species variability to the regressions.

Results and Discussion

In the present study ECOSAR overestimated the toxicity for 70% of the 59 compounds where both measured and modelled data exists. For the remaining 30% more than 94% of the predictions underestimated toxicity by less than a factor of ten. This is a first line of evidence that a narcosis based model approximately 70% of the time is conservative relative to experimental values, thus implying that for at least 70% of the APIs their acute MOA can be explained by baseline toxicity (Fig. 1). Compounds with a predicted EC_{50} > 2 × standard error of prediction (SEP) can be assumed to have a substantial contribution other MOAs than narcosis (Öberg 2004). An excess toxic ratio (Te) between predicted and measured EC_{50} (Russom et al. 1997) >7 = 2 × SEP (Öberg 2004) and are thus indicative of chemicals behaving according to some kind of specific MOA. In this study we found that 85% of the APIs had Te's <7 [overall mean Te = 6.8 (±22.4 SD)]. Nine APIs had Te's >7: Metformin (10.3); Paroxetine (14.3); Digoxin (18.5); Nitroglycerin (19.3); Carvediol (24); Lomefloxacin (28.1); Propranolol (35.9); Warfarin (53.3); and Niacin (162.1).

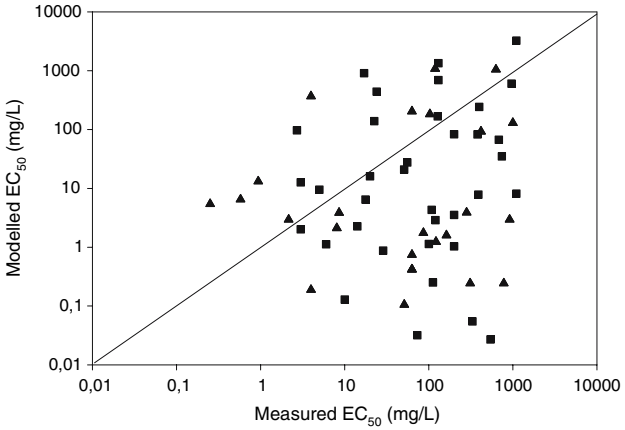

Fig. 1 Sensitivity evaluation of ECOSAR predictions (Sanderson et al. 2004) and measured data from NOAA (2006) for fish (*squares*) and daphnids (*triangles*) (n = 59)

There is a strong correlation between all the modelled Log EC_{50} values and Log K_{ow}, with correlation coefficients (r^2) ranging from 0.73 to 0.76. The Log EC_{50}–Log K_{ow} slopes of the regressions range from −0.5 to −0.65, which is at the tail end of the range for the universal narcosis slope range, suggesting that for the majority of APIs the primary MOA, at least for fish and daphnids, is narcosis (II) (slope = −0.65) (Russom et al. 1997) (Fig. 2a–c). The slopes of the Log EC_{50}–Log K_{ow} regressions based on

Table 1 (Q)SAR models for APIs

Log EC_{50} (mg/L)	Slope (Log K_{ow})	Intercept	r^2
Model fish	−0.65	2.37	0.73
Model daphnids	−0.60	2.06	0.75
Model algae	−0.49	1.77	0.76
Measured fish	−0.86	2.87	0.26
Measured daphnids	−0.86	3.12	0.16

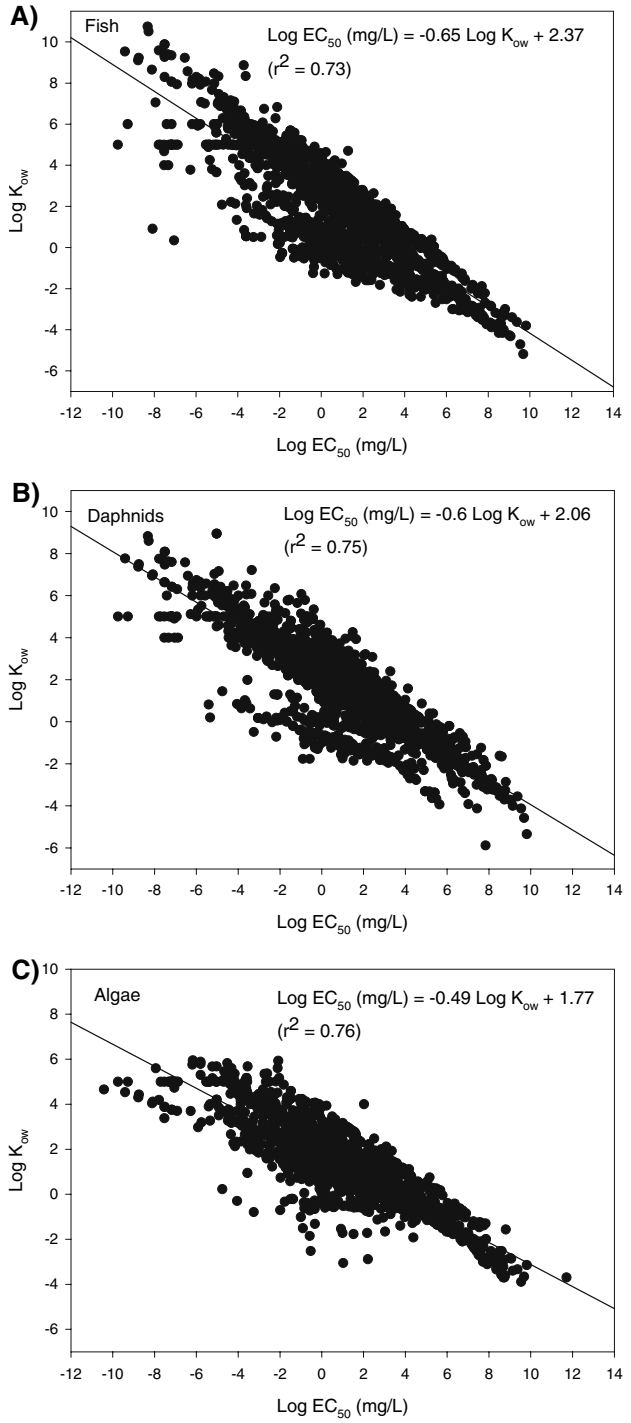

Fig. 2 **a–c** Log K_{ow} based (Q)SAR from modelled toxicity values (n = 2,575) (Sanderson et al. 2004)

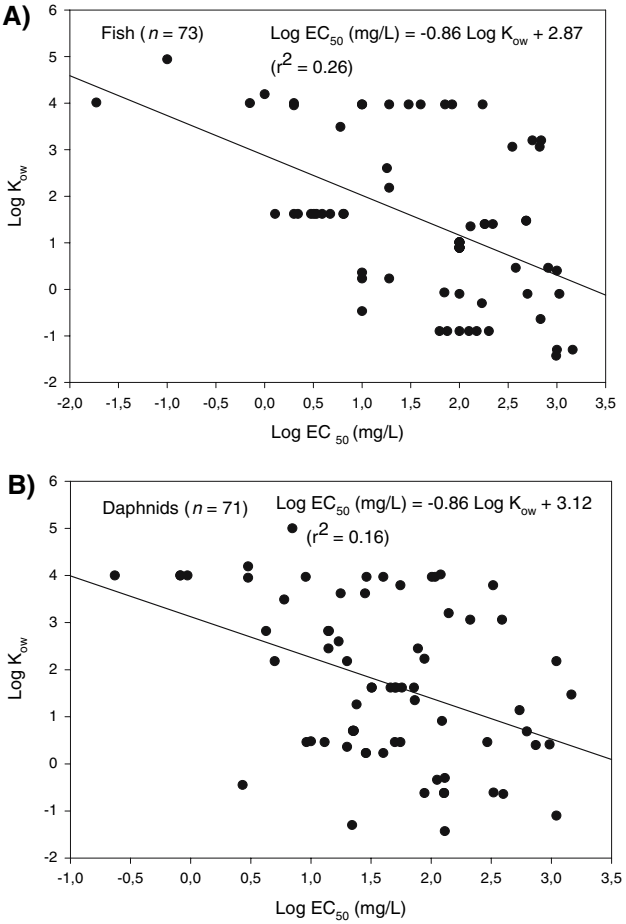

Fig. 3 **a–b** Log K_{ow} based (Q)SAR from measured toxicity values (NOAA 2006)

measured data from the NOAA (2006) database for both fish and daphnids equals −0.86 thus suggesting a narcotic MOA. However, due to, e.g. inter-laboratory noise and inter-species extrapolation uncertainty the correlation coefficients are only 0.26 and 0.16, respectively, limiting the weight of these lines-of-evidence (Fig. 3a–b). From an overall comparison, despite relatively weak correlations, the measured Log EC_{50}–Log K_{ow} regressions are more dependent upon Log K_{ow} (steeper negative slope), than the ECOSAR based Log EC_{50}–Log K_{ow} regressions (Table 1).

It is not surprising that the r^2 in Table 1 is lower for the measured data due to the limited number of data points and the inherent measurement variability, however, it is somewhat surprising that the slope is greater (greater Log K_{ow} dependence) for the measured data than for the modelled data.

Based on the above lines-of-evidence, as well as the findings by Escher et al. (2002, 2005), it seems that the vast majority of APIs acute aquatic toxicity can be attributed to a narcotic MOA. However, as Seiler (2002) pointed out there are multiple potential overlaps between APIs mammalian pharmacodynamic MOA and potential non-target aquatic organisms effect parameters, so why do these known and unknown MOAs not manifest themselves when we assess the toxicity of APIs?

One answer could be found in the "Lamppost analogy"; *looking for the car keys under the street lamppost – even*

though they got lost by the buildings front door in the dark. Likewise, our current standardized laboratory ecotoxicity tests are apparently not selective of potential subtle pharmacodynamic and specific MOAs of APIs. A survival test with duration of three weeks is probably rarely enough time for an organism to become statistically significantly impaired or die from sublethal effects. In other words, current standardized tests are primarily tests of lethality rather than toxicity, hence narcosis, or baseline lethality, as a function of the compounds Log K_{ow} is the dominant descriptor. Most of the standardized test used today for registration of chemicals were developed during the 1970s and 1980s and adopted in international guidance documents by the Organisation for Economic Co-operation and Development (OECD) during the mid 1980s (http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1,00.html). The environmental problems and concerns at that time were primarily relative to measured environmental exposures of less polar chemicals typically in the mg/L levels. Hence, the majority of current standardized toxicity tests were tailored towards effect estimates in the mg/L range. However, the current context and types of concerns about APIs are quite different (Daughton and Jones-Lepp 2001). With increased accessibility of more sensitive and selective analytical capabilities during the 1990s more polar compounds and mixtures at ng/L levels were more readily measured in the environment and reported in the literature. The United States Geological Survey (USGS) study by Kolpin et al. (2002) is a prime example of how recent feasible large scale analytical works received significant attention regarding APIs in the environment. Given the continual and low level environmental exposure (ng/L) of APIs (Kolpin et al. 2002; Daughton and Jones-Lepp 2001) and the orders of magnitude between these and acute effect concentrations (Sanderson et al. 2003), assessment of long-term toxicity, potentially causing subtle impairment of the organism through the ecosystem at the ng/L level, seems more relevant in a risk assessment context than assessment of acute toxicities at the mg/L level for most APIs.

Relative to environmental realism of APIs toxicity it is important to realize that a compound may have several different adverse MOAs dependent upon, e.g. the type and sensitivity of exposed organism, internal exposure concentration at various target sites and presence of other compounds (Luckenbach and Epel 2005). The extrapolation from laboratory based narcotic lethal concentrations of APIs in the mg/L range to environmentally relevant concentrations in the ng/L range is uncertain. The predominating threshold dose-response assumption has been challenged with mounting evidence of hormetic (*J*-shaped) dose-response relationships (Calabrese et al. 2006) suggesting that compounds might not have a threshold triggered baseline/minimal toxicity level primarily described by their Log K_{ow} at environmentally relevant concentrations. In other words, subtle effects would be expected to occur at low concentrations (ng/L) of APIs. Conversely, various inter- and intracellular communication and defense/repair mechanisms, e.g. gap junctions, support homeostasis and thus decrease the expression of toxicity at low concentrations (Ruch 2002).

With progression of our knowledge of pathology, the cell membrane, intracellular signalling pathways, and toxicogenomics it is evident that the protein affinity, size and shape of toxicants are crucial for crossing the cell membrane and expression of the compound's potential excess toxicity. Ignoring these properties by focusing on acute lethality elicited by non specific disruption of lipids in the cell membrane as a function of compounds hydrophobicity in the laboratory and subsequently in modelling is not sufficient to describe APIs ecotoxicity, when it is reasonable to expect specific MOAs (Seiler 2002). Similarly, specific toxicological properties relative to emerging contaminants can be expected, e.g. for nanomaterials relative to their size and unique surface properties.

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