

*39th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals,
Pesticides and Biotechnology
15-17 February 2006*

**Focus Session: Experiences using integrated approaches to fulfil information requirements
for Testing and Assessment**

**Contribution by Denmark
(Examples on Integrated Information Strategies on untested chemicals used for selected
regulatory purposes)**

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Danish EPA	Ministry of Environment
<p>OECD Joint Meeting (Febr. 2006) Special Session on <i>Integrated Approaches to Testing and Assessment</i></p> <p>Examples on Integrated Information Strategies on untested chemicals used for selected regulatory purposes.</p> <p>Henrik Tyle, Chemical Division, Danish EPA</p>	

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<p>The two selected examples:</p> <ul style="list-style-type: none">• The Advisory Self-classification List for untested chemicals• Screening for PBT candidates <p>in both cases: test data have not been considered - <i>QSAR based approaches</i> based on work by <i>Jay Niemelä & Eva Wedebye</i>, DFVF (Danish Food Research)</p>
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**DK-EPA "Advisory Self-classification list
regarding untested chemicals" (2001)**

Background:

- for chemicals with no harmonised EU classification: *self-classification* according to the EU Labelling Guide is *an obligation*
- *often* very little/*no test data* available on chemicals
- *DK EPA QSAR database* had been established:
 - 70 QSAR equations/ models/ endpoints
 - P/C & environmental fate properties, toxicity and ecotoxicity endpoints
 - contains predictions on 166.000 discrete organic chemicals
 - various search facilities (CAS no, sub-structures, names etc.)
 - half of all EINECS chemicals covered (excl. inorganics and multi-constituent chemicals)

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**DK-EPA "Advisory Self-classification list
regarding untested chemicals"**

Problem:

Often users of chemicals cannot discriminate between
"not classified" and *un-classified*" for hazardous properties:

- **not classified:** *evidence for absence* of dangerous properties
i.e. sufficient data indicate lack of hazardous properties
- **un-classified:** *absence of evidence* of dangerous properties -i.e. no data for considering and classifying for hazardous properties.

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**DK-EPA "Advisory Self-classification list
regarding untested chemicals"**

Solution to the problem:

- *EU Labelling Guide* Introduction: *use of structure-activity and expert judgement* can in general be used for hazard classification purposes
- DK EPA QSAR experts: *(Q)SAR predictions* are now in many cases so reliable that they *can be used for classification purposes*, when no test data are available

⇒

DK EPA could in a practical way *help self-classifiers* to fulfil their obligation - *also on the many of the un-tested chemicals / endpoints*

the alternative was to keep on accepting that hazard classification & labelling often is NOT done only because of lack of test data!
- and continued confusion of chemical users.

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**DK-EPA "Advisory Self-classification list
regarding untested chemicals"**

Basic concept:

- *match QSAR model endpoints with classification endpoints*
 - combination of QSAR models, i.e. *model algorithm: match with classification criteria & minimise no of false positives (specificity, i.e. not focused on maximising sensitivity)*
- ⇒ non-tested potentially dangerous chemicals/ properties may "slip through", so the list cannot be used for claims of no danger or "no classification" for non-tested chemicals (also because the AD of the models do not cover all EINECS chemicals for which predictions were made)
- ⇒ but nevertheless *a lot of proposals for self-classification could be made & the number of un-classified chemicals could be reduced this way*

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DK - EPA "Advisory Self-classification list regarding untested chemicals"

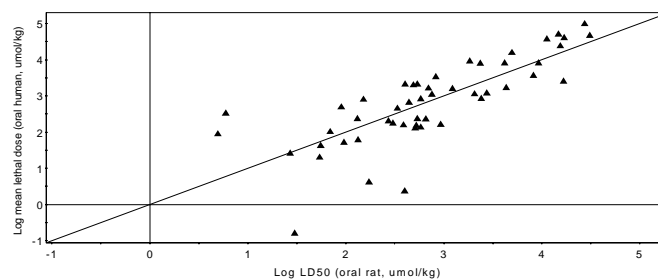
"Positive" QSAR predictions used in relation to:

- *acute oral tox (R22) - cf. 1st example* ⇒
- *sensitization by skin contact (R43)*
- *mutagenicity (R40) -*
- *carcinogenicity (R40) - cf. 2nd example* ⇒
- *danger to the environment; aquatic hazard (R50, R50-53, R51-53 & R 52-53) - cf. 3rd example* ⇒

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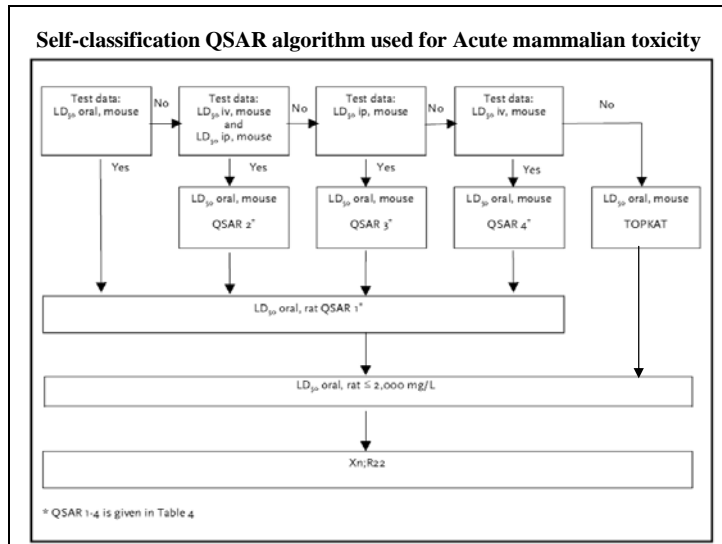
Correlation between oral rat data and human toxicity

Prediction of human average mean oral lethal dose from rat oral LD50
 $y=0.829 \cdot 0.877$, $r^2=0.607$, W alum 1998, EHP Suppl., Vol. 6, No. 2, 497-503

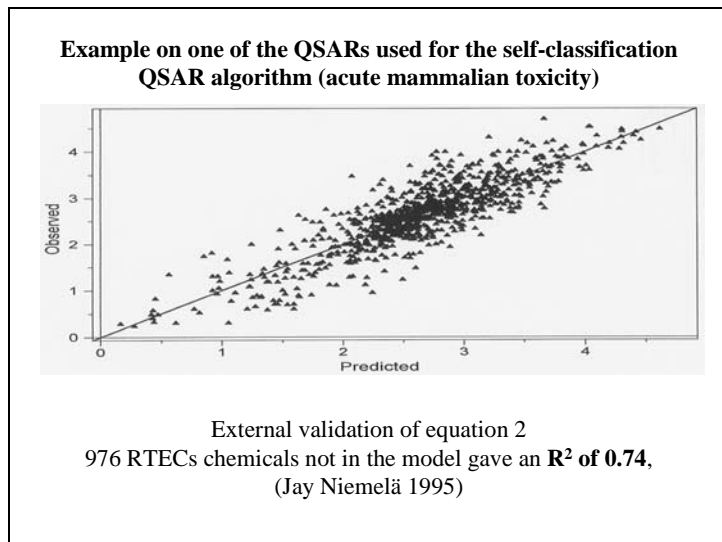


This type of observed correlation ($R^2 = 0.6$) is the basis for the current regulatory acceptance in EU & GHS of "the rat model" for the **acute toxicity classification criteria** for human health protection !

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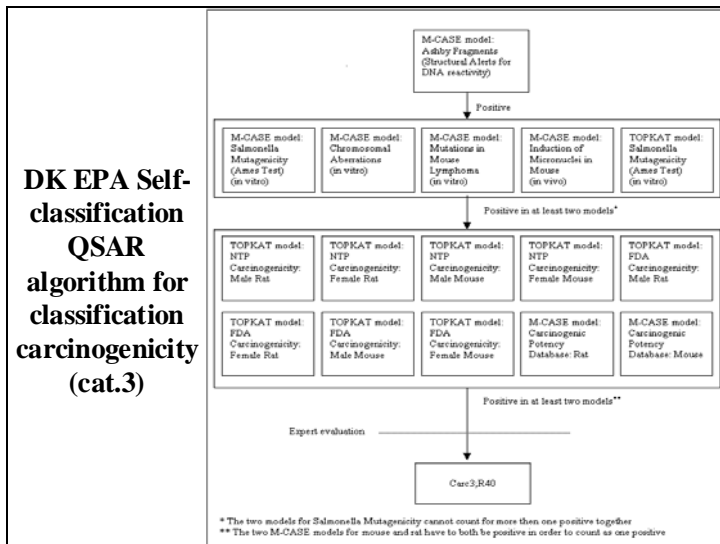


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DK - EPA Self-classification QSAR algorithm for environmental (aquatic) hazard classification

- **Predicted data:**
 - **Aquatic toxicity:**
 - Log Kow < 6: *MCASE fathead LC50-minnow model (DK-EPA)*
 - Log Kow > 6: *acute minimum fish tox (QSAR recommended in EU TGD)* & decreasing toxicity the higher log Kow (according to BCF-WIN)
 - **Degradability:** *BIOWIN1 model* (very high probability of NRB predictions to be correct but only half of them identified)
 - **Bioaccumulation:** *BCFWIN-model* (exclusion of extremely lipophilic chemicals and types of chemicals with low bioaccumulation)
- **Advisory Environmental Hazard classifications:**
 - the classification criteria (classification endpoints and cut off values) were used directly
 - *the used QSAR model algorithm: lowers the possibilities for “over-classification”*

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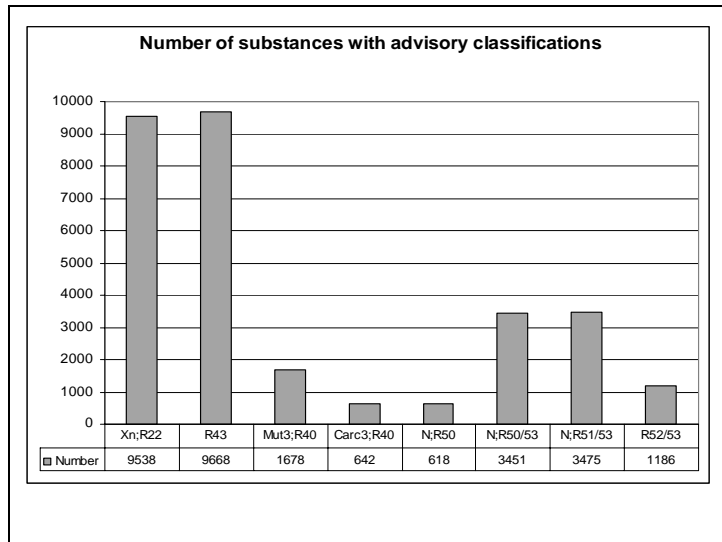
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DK-EPA self-classification algorithm for carcinogenicity relative to the classification criteria

- The QSAR model algorithm in short:
 - structural alerts for *genotoxicity* AND
 - at least *two positive* in vitro / in vivo *mutagenicity test predictions* AND
 - at least *two positive carcinogenicity bioassay predictions*
- The classification criteria (EU cat. 3/ GHS cat.2) shortly:
 - limited evidence from human or animal carcinogenicity studies

⇒ *the used QSAR model algorithm lowers the possibilities for “over-classification” in relation to the true carcinogenicity potential* (e.g. no identification of non genotoxic carcinogens)

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How to use the Advisory Self-classification list ?

- *Step 1:* Use the “ *EU List of Dangerous Substances*”
- *Step 2:* If the substance is not here, *classify* on the basis of data from experimental tests, experience, (Q)SAR and expert judgements following the *specific criteria* in Annex VI (*EU Labelling Guide*)
- *Step 3:* If no data are available, *classify* on the basis of the *Advisory list for self-classification* of dangerous substances, Danish EPA
- **Published in 2001:** www.mst.dk/chemi/01050000.htm
- *work in progress at DFVF for DK EPA for updating of the list*

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Screening for candidate PBTs

- Started in 2001: UK, Nordic, ECB and D exercise on HPVCs (> 1000 tpa/producer) mostly based on test data
- DK-EPA QSAR exercise on H- & MPVCs (>10tpa/producer)
- We proposed screening criteria based on combination of QSAR model predictions
- they are referred to in the current TGD for expert case by case use on non-tested chemicals / endpoints

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DK EPA Screening for candidate PBTs by use of QSARs

- **Persistence:** 3 models in an algorithm predicting not ready biodegradability (BIOWIN 2 & 6) AND long environmental half life (BIOWIN 3) \Rightarrow P, and then
- **Bioaccumulative:** 2 models regarding BCF in fish:
 - BCFWIN $>$ 2000 \Rightarrow B or
 - BCFWIN $<$ 2000 but BCF(Connell) $>$ 2000 AND positive expert judgements (e.g based on hindrance for uptake due to molecular dimension and the potential for metabolism)

\Rightarrow **un-tested PB-candidates**

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PB(T) screening by use of QSARs: Further evaluation: environmental release potential.

- No of substances ($>$ 10 tpa/ EU producer) evaluated: 5716
- Total no. of potential PBTs/vPvBs as identified by QSARs: 134
- No. registered in *Nordic Product Registers*: 66
- **No with possible release due to widespread use in products: 16-32**
(depending of the definition of significant environmental release potential)

excluded: PBT/vPvB candidates released from (industrial) processes & multi-constituent chemicals (mixtures), nevertheless:

\rightarrow *no. of relevant PBT/vPvBs is relatively small according to this QSAR based screen*

\rightarrow *inclusion of PBTs/vPvBs under an authorisation scheme in REACH seems feasible based on the QSAR screening and the Product Register information*

\rightarrow *selected HPVCs were submitted to the EU PBT WG & QSAR predictions used case by case based on expert judgement together with available - sometimes scarce test data (also used on all other selected PBT candidates, including some multi-component chemicals)*

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Danish EPA	Ministry of Environment
Concluding remarks	
Acceptability of (Q)SAR / (Q)SAR predictions is context dependent:	
<ul style="list-style-type: none"> - test data vary - and individual test data may be uncertain - (Q)SAR predictions may guide in evaluation of available test data - and increase knowledge regarding not tested chemicals and endpoints - normally weight of evidence including more than one prediction will be 	
used as:	Influence on assessment and decision making:
1) <i>Alternative to test</i> ⇨	<i>increases</i> uncertainty
2) <i>Alternative to no test</i> ⇨	<i>decreases</i> uncertainty
3) <i>Supplement to test</i> ⇨	<i>decreases</i> uncertainty by enhancing robustness of assessment
Scenario 2) most frequent - also after entering into force of REACH	

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Danish EPA	Ministry of Environment
Concluding remarks	
<ul style="list-style-type: none"> - when no/limited test data: consequence of <u>not</u> accepting non-test /(Q)SAR information : <ul style="list-style-type: none"> • Triggering of test data & decision based on this ? - or no test data and hence no decision possible ? - what is the acceptability of this in each case ? - What type of Regulatory use? Priority setting for testing, C&L, limit value, risk assessment potentially leading to risk management ? - when predicting <i>regulatory "endpoints"</i> match predictions of different QSAR models to "woe" rules or "criteria" of the regulatory framework - (normally related to more types of (test) data used in a prescribed way) 	

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Danish EPA	Ministry of Environment
Concluding remarks	
<i>In relation to use of QSAR models consider:</i>	
– the 5 OECD scientific validation principles including the <i>performance</i> of the individual (Q)SARs / QSAR battery algorithm	
– but <i>relative to regulatory acceptability</i> e.g.	
<ul style="list-style-type: none">• are false positives as acceptable as false negatives for the particular regulatory decision ? - may depend on testing requirements and default decision rules• what is the <i>required</i> accuracy/confidence in relation to the regulatory cut off point ? (e.g. what is the distance of a predicted value from the regulatory cut off / trigger values ?)	

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APPENDIX: Supplementary information not included in the oral presentation:
<ul style="list-style-type: none">• concerning the DK-EPA database (cf. also a separate presentation)• QSAR algorithm used for environmental (aquatic) hazard classification• various details regarding some of the QSAR models used for self-classification• performance of some of the QSAR models used for self-classification

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Validation status regarding the QSAR models of the DK EPA database

- **MCase-models:** only predictions within applicability domain as defined by MCase & with no warnings regarding unknown fragments accepted. (AOK marked in database)
- **DK-EPA models (in-house MCase models):** typically cross validated LGO 10 or 50 % out, when possible also externally validated (cf. overview with validation results on DK EPA home page for the Advisory Self-class. List - typical performance: sens.: 70-80%, specificity: 80 %)
- **TOPKAT models:** only predictions within appl. domain as def. By TOPKAT & with 4 closest analogs also predicted OK accepted
- **Other models /equations:** EPIWIN and TGD equations; typically not possible in an easy way to generally describe validation status (but reference to source ref./ data/ inst.)

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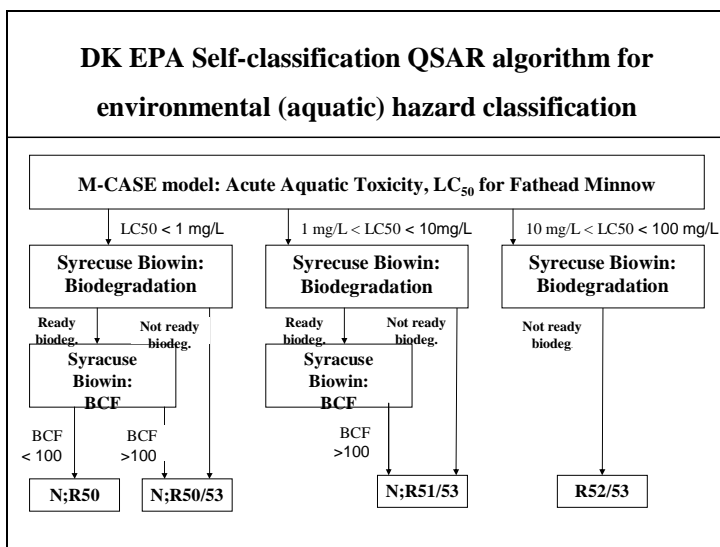
4 of the QSARs used for self-classification QSAR algorithm for acute mammalian toxicity)

1. **Log LD50 (oral, rat) = 0.731 + 0.841 (log LD50 (oral, mouse))**
RTECS 1989, n = 3919, R² = 0.770, Q² = 0.749
2. **Log LD50 (oral, mouse) = 0.682 + 0.373 (log LD50 (iv, mouse) + 0.518(log LD50 ip, mouse))**
RTECS 1994, n = 286, R² = 0.766, Q² = 0.764
3. **Log LD50 (oral, mouse) = 0.731 + 0.841 (log LD50 (ip, mouse))**
RTECS 1994, n = 286, R² = 0.724, Q² = 0.724
4. **Log LD50 (oral, mouse) = 0.945 + 0.802 (log LD50 (iv, mouse))**
RTECS 1994, n = 286, R² = 0.689, Q² = 0.688

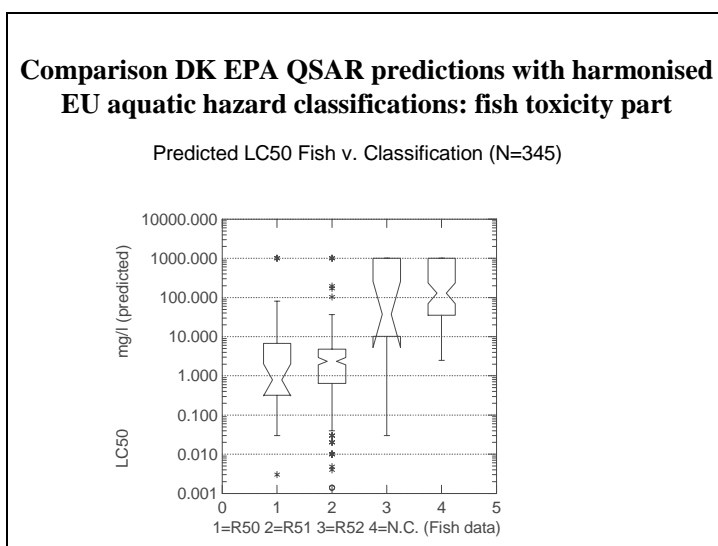
TOPKAT
External validation of Rat Oral Toxicity Acute model

Result predicted within a factor of +/-:	%	N (cumulative)
2	42	671
4	67	1,069
6	78	1,235
8	83	1,323
10	86	1,368

In modern acute tox tests using small numbers of animals, statistical variation is often within a factor of 2-4, and inter-laboratory variations of up to 10 are not uncommon



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Acute fish toxicity estimated for self-classification

Chemicals with log Kow < 6:

MCASE QSAR model:

- Training set: 549 high quality fish LC50 (96 hrs) (USEPA)
- Performance: LGO(10%): $R^2 = 0.74$

Chemicals with log Kow > 6 (minimum tox at steady state):

Pseudo-LC50 for LBB (non-polar narcosis) at steady state:

$$BCF = C_{biota}/C_{water} \Rightarrow C_{water} = C_{biota}/BCF$$

LBB for non-polar narcosis: 8.15 mmol/L

i.e. $LC_{50} = 8.15 \text{ mmol/L} / BCF - WIN$

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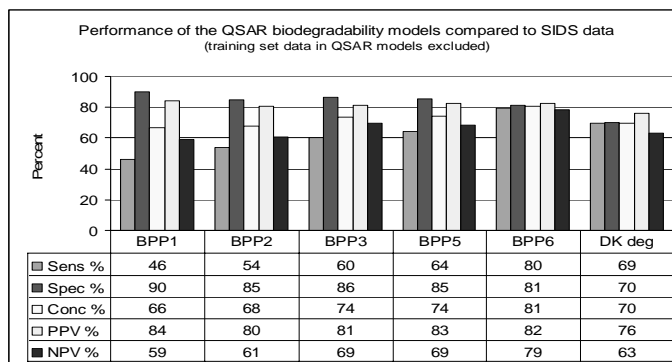
Comparison DK EPA QSAR predictions with SIDS test data: biodegradability part

- Comparison of agreed SIDS test data with QSAR model predictions (cf. OECD doc.: ENV/JM/TG(2004)26Rev1):
 - QSAR models: **BIOWIN 1,2,3,5,6** and DKEPA model
 - Previously validated in relation to cut off points for prediction of ready and not ready biodegradability
 - This comparison: 164 chemicals:
 - 84 tested "ready" and 80 "not ready" according to SIDS test data (optimal for validation)
 - exclusion of chemicals represented both in training set of models and in SIDS data (=> external "validation")
 - DK-EPA (MCase)-model: also excl. of predictions outside AD
- **NRB = "positive" (fraction predicted correctly: sensitivity),**
RB = "negative" (fraction predicted correctly: specificity)

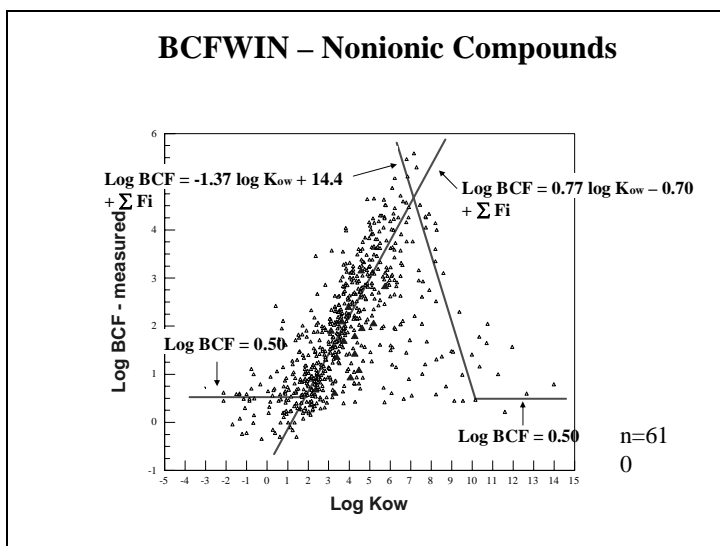
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Comparison DK EPA QSAR predictions with SIDS test data: biodegradability part:

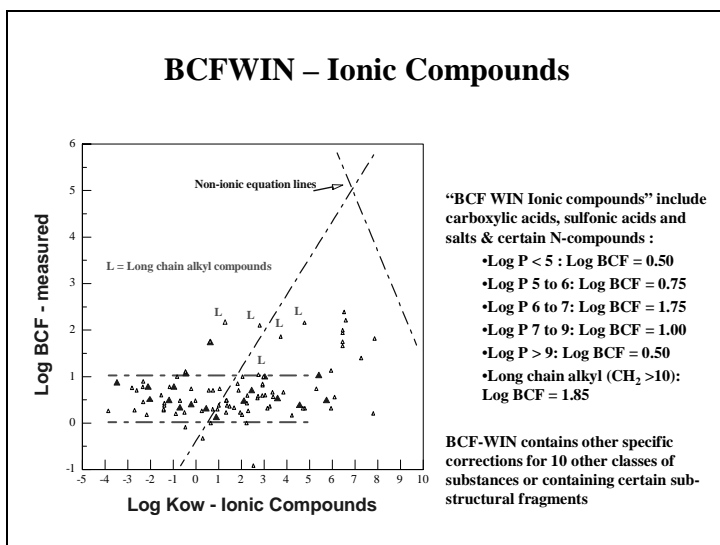
- *BIOWIN 1 (BBP1)* was used for Self-classification: 1) many NRBs not identified (sens. - blue), but 2) very high probability of NRB predictions to be correct (PPV - light blue)



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Comparison DK EPA QSAR predictions with harmonised harmonised EU hazard classifications for carcinogenicity

- 762 chemicals on Annex I were included in the systematic screening of the ~47,000 Eines substances
- Of these 89 were classified for cancer
- Robust predictions for 55, i.e. 62% within the A.D. of the QSAR models which according to the classification QSAR algorithm is the minimum for classification
- Of these 46 were predicted to be carcinogens
- i.e. *sensitivity* (fraction of positives predicted positive) is $46/55 = 84\%$
- $762 - 89 = 673$ substances were not classified for cancer on Annex I
- Of these, 63 substances were predicted carcinogenic by the QSAR battery algorithm
- Assume also domain of 62% for the not classified substances: 417 substances
- Means that *specificity* (fraction of negatives predicted negative) is $(417 - 63)/(417) = 85\%$
- *Are some of the substances on EU's List of Dangerous Substances not classified for carcinogenicity because of lack of sufficient test data on this endpoint? Most probably - e.g. a range of aromatic amines among the 63 not classified substances*
- $PPV = 46/(46+63) = 42\%$ $NPV = 417/(417+55-46) = 98\%$