

Eminent Toxicologist Lecture Series

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Society of Toxicology

Eminent Toxicologist Lecture Series

QSARs to Commemorate the Fiftieth Anniversary of the RD50

Annual Society of Toxicology Meeting

March 2016

* No conflict of interest in the preparation and presentation of these slides

Society of Toxicology

OBJECTIVES AND GOALS

Estimates of Threshold Limit Values (TLVs)
for New Chemicals

- Restricted to Volatile Organic Chemicals (VOCs)
- Both Non-Reactive (nrVOCs) and Reactive (rVOCs)
- Estimates obtained from RD50 values obtained in mice

- Estimates obtained from computational (calculation) methods capable of estimating physicochemical descriptors.
- Be in a position to estimate the potency of new and never-tested chemicals if the computational methods are verified, and if so:

“Estimates of TLV, Not RD_{50} , for New Chemicals is What We Want”

BRIEF HISTORY - TRIGEMINAL REFLEX

Original article: Kratschmer, F., 1870.

Über reflexe von Nasenschleimhaut auf Athmung und Kreislauf.

SBer. Akad. Wiss. Wien Math. Naturwiss. K1.62, 147-170.

Über Reflexe von der Nasenschleimhaut auf Athmung und
Kreislauf.

Von Dr. F. Kratschmer,

k. k. Oberärzte und Assistenten am physiologischen Institute der Josefs-Akademie.

(Mit 2 Tafeln.)

Die Reflexe, welche bei Reizung der Nasenschleimhaut ausgelöst werden, sind bis jetzt noch nicht Gegenstand einer besonderen Untersuchung gewesen.

ELSEVIER

Respiration Physiology 127 (2001) 93–104

Original article: Kratschmer, F., 1870. Über reflexe von Nasenschleimhaut auf Athmung und Kreislauf. SBer. Akad. Wiss. Wien Math. Naturwiss. K1.62, 147-170. Translated by Elisabeth Ullmann.

Translation of an historic article:

On reflexes from the nasal mucous membrane on respiration
and circulation ^{1, ☆}

F. Kratschmer

**Recherches sur les actions réflexes
produites
par l'irritation des voies respiratoires**

par H. MAGNE, André MAYER, L. PLANTEFOL

Ann. Physiol. 1: 394-427, 1925

**EFFECT OF VARIOUS INHALED VAPORS ON RESPIRATION
AND BLOOD PRESSURE IN ANESTHETIZED, UNANES-
THETIZED, SLEEPING AND ANOSMIC SUBJECTS**

WILLIAM F. ALLEN

From the Department of Anatomy of the University of Oregon Medical School, Portland

Amer. J. Physiol. 88: 117-129, 1929

The subjects of these experiments were normal individuals, an anosmic and selected hospital patients under light ether anesthesia. The normal subjects and the anosmic were students who had done some laboratory

*Reprinted from the Archives of Environmental Health
October 1966, Vol. 13, pp. 433-449
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Irritating Properties of Airborne Materials to the Upper Respiratory Tract

YVES ALARIE, PhD, FALLS CHURCH, VA

THERE is a need for a suitable methodology by which the irritating properties of compounds in an airborne form can be assessed. Present methods, based upon histopathological examination of tissues follow-

ing compounds for their possible irritating effects is based upon reflex-induced modifications of the respiratory pattern following administration of aerosols or vapors. The modifications of the respiratory pattern in animals

PRIOR WORK AS A STARTING POINT

Sensory irritation is a frequently-used basis for TLVs; from 25% to 50% of TLVs have sensory irritation as a direct or secondary part in their basis.

The potency of VOCs as sensory irritants has been evaluated in mice since 1966* and:

*From: Alarie, Y. (1966). Arch. Environ. Health 13: 433-449

- a) formal validation was undertaken using 26 positive and 26 negative sensory irritants in humans, resulting in the same classification in mice.

- b) the potency of sensory irritants in mice was then measured from a decrease in respiratory frequency by 50% (RD_{50}) using concentration-response relationships.

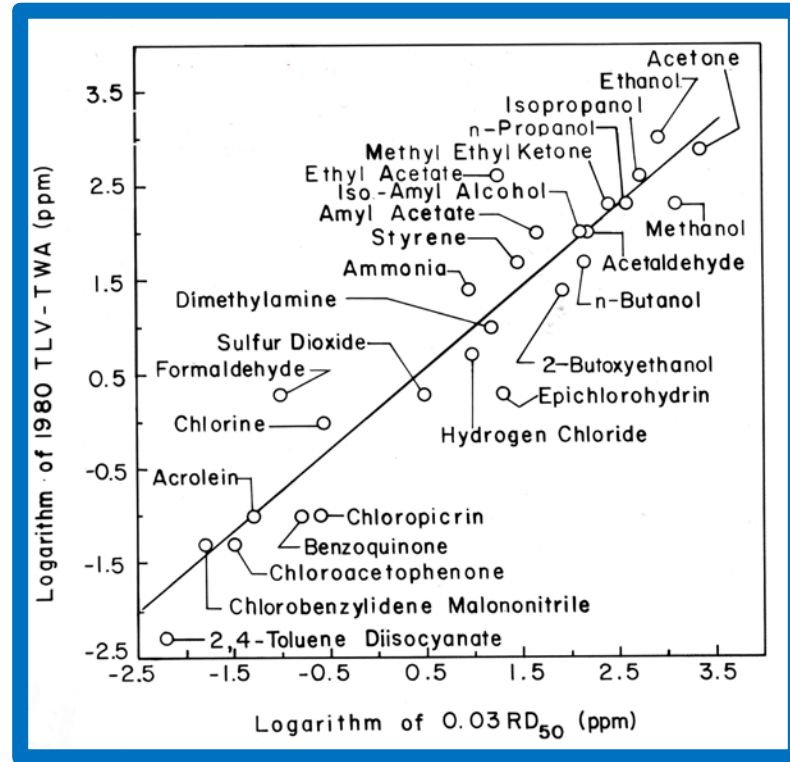
- c) it was suggested that at $0.03 \times RD_{50}$, minor sensory irritation would be observed in humans and this could be used to establish a TLV and regression analysis was tried.
- d) an excellent linear regression relationship ($r^2 = 0.89$) using 26 chemicals was published in 1980.

This relationship, between $0.03 \times RD_{50}$ and TLV, is shown on the next slide.

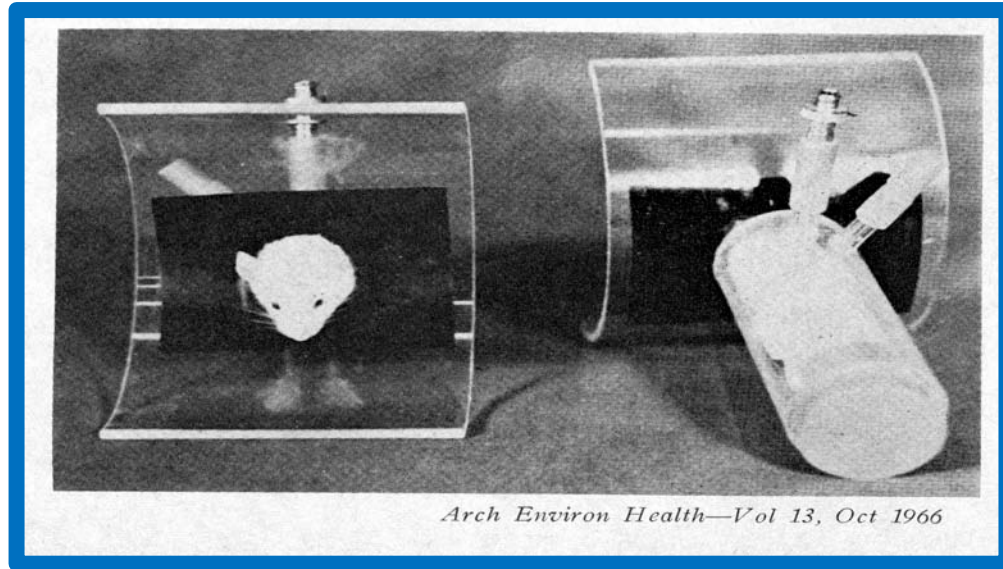
Alarie, Y. (1980). Food and Cosmetics Toxicology 19: 623-626.

Alarie, Y. (1981). Environmental Health Perspectives (EHP) 42: 9-13.

26 Chemicals
R sq. = 0.90



BRIEF DESCRIPTION OF THE ORIGINAL RD₅₀ METHOD, “THE MOUSE BIOASSAY”

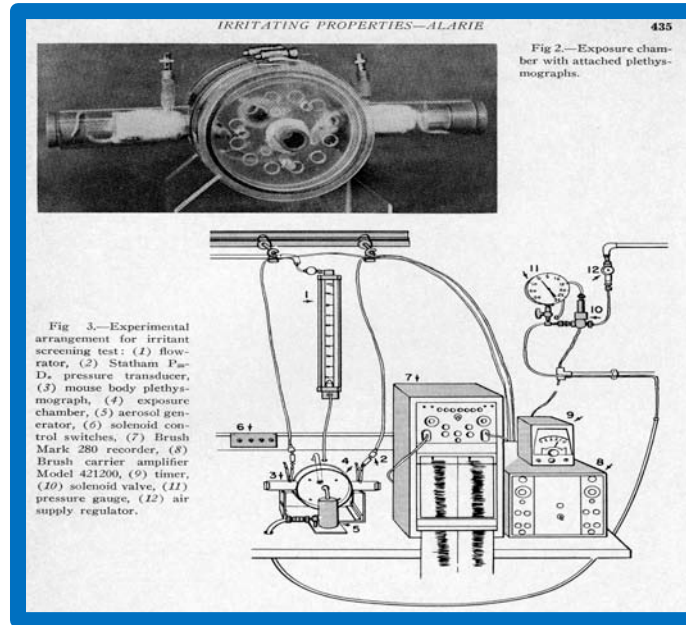


From: Alarie, Y. (1966). Arch. Environ. Health 13: 433-449

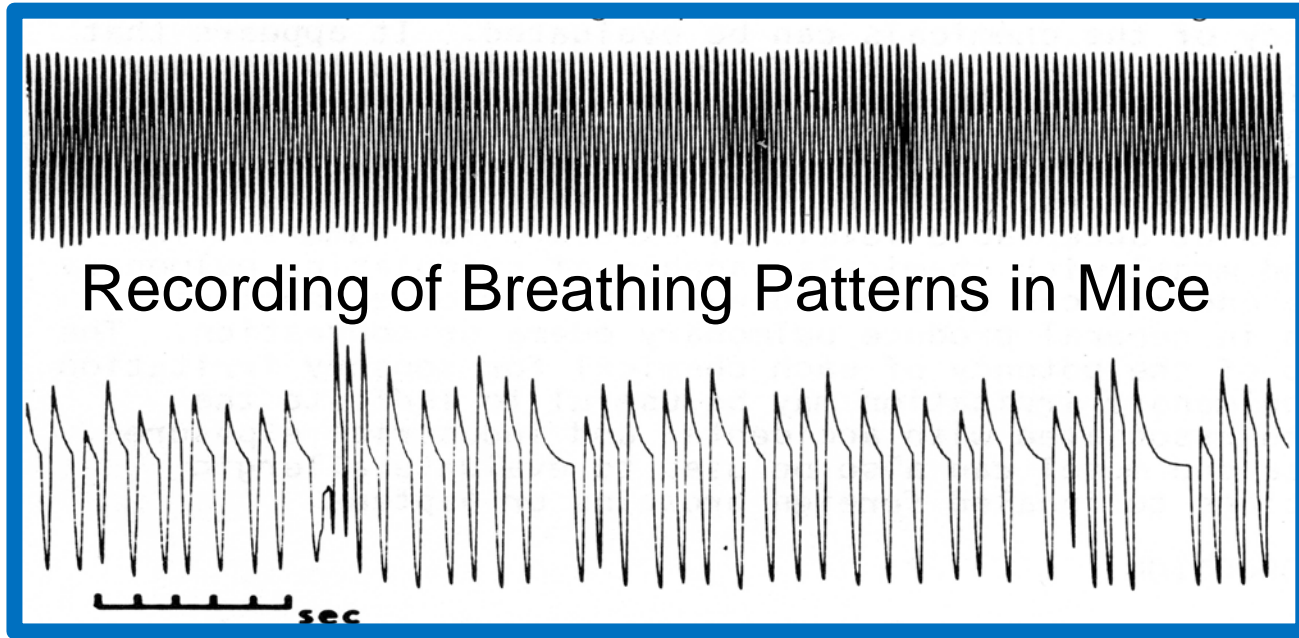
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BRIEF DESCRIPTION ...

Exposure Chamber



BRIEF DESCRIPTION ...



BRIEF DESCRIPTION ...

Concentration-Response Relationship and RD_{50}

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IRRITATING PROPERTIES—ALARIE

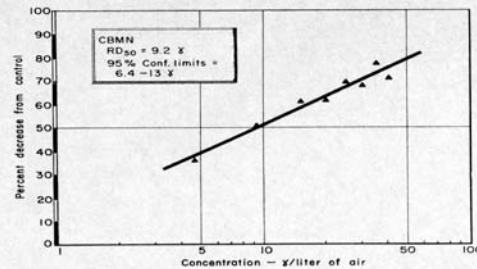


Fig 13.—Dose-response evaluation of peak percent decrease in respiratory rate, regardless of the time period at which it occurs during or following exposure to CBMN.

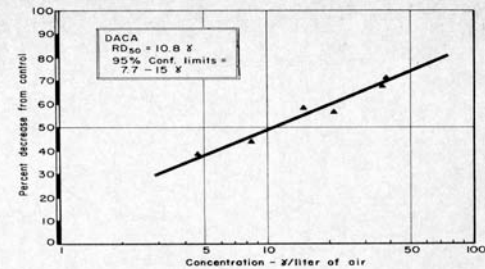


Fig 14.—Dose-response evaluation of peak percent decrease in respiratory rate, regardless of the time period at which it occurs during or following exposure to DACA.

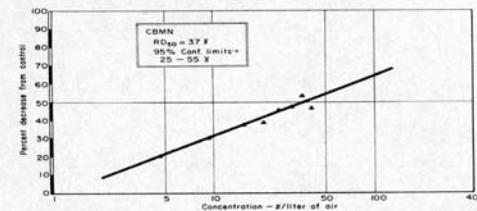


Fig 15.—Dose-response evaluation of mean percent decrease in respiratory rate during the exposure period to CBMN.

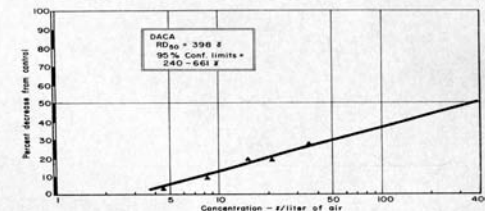


Fig 16.—Dose-response evaluation of mean percent decrease in respiratory rate during the exposure period to DACA.

COMPUTERIZED METHOD TO EVALUATE SENSORY IRRITATION (SI), AIRFLOW LIMITATION (A), AND PULMONARY IRRITATION (P): SI, A, P

The next set of three slides (1, 2, 3) was taken from:
Vijayaraghavan, R, Schaper, M, Thompson, R, Stock, MF, Boylstein, LA, Luo, JE and Alarie, Y. (1994).
Computer assisted recognition and quantitation of the effects of airborne chemicals acting at different areas of the respiratory tract in mice. Arch. Toxicol. 68: 490-499.

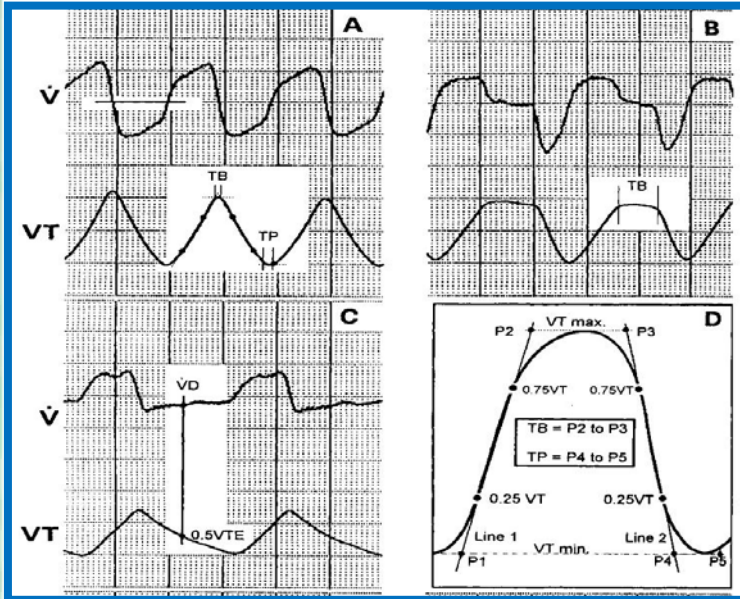
1) COMPUTERIZED METHOD FOR SI, A, & P

A: Normal conditions

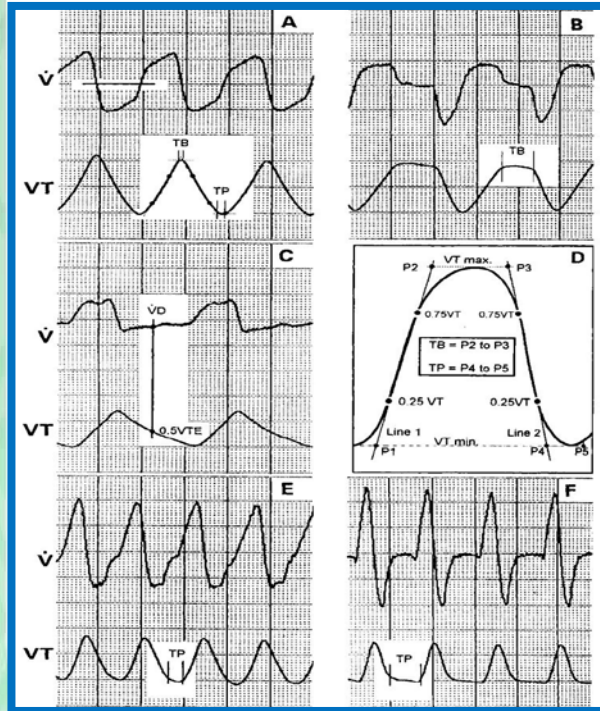
Airflow signal (\dot{V}) and integrated \dot{V} to yield V_T . A horizontal line is drawn at zero \dot{V} , separating the airflow during inspiration (V_I), upward and airflow during expiration (V_E), downward.

D: Measuring TB and TP

Hand-drawn wave showing how TB (duration of braking) and TP (duration of pause) are measured.



2) COMPUTERIZED METHOD FOR SI, A, & P



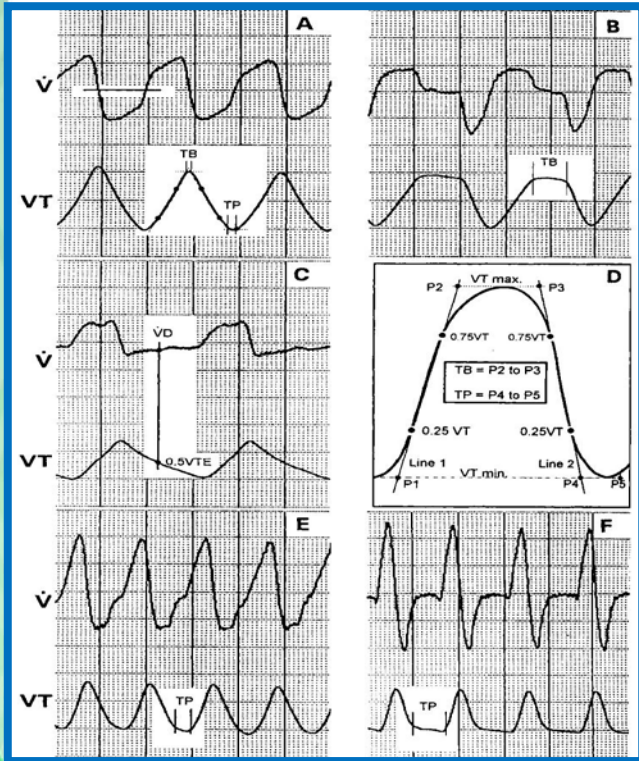
B: During sensory irritation

Note the major change: an increase in TB. This will also result in a net decrease in respiratory frequency (f).

C: During bronchoconstriction

Note the major changes: longer duration of expiration with much lower \dot{V} than normal. To quantify this change, \dot{V} at mid-tidal volume during expiration (0.5 VTE), abbreviated VD here is measured. VD has also been abbreviated EF50, expiratory flow at 0.5 VT . There is also a net decrease in f .

3) COMPUTERIZED METHOD FOR SI, A, & P



E: During pulmonary irritation

Note the major changes: VT is lower and f is higher (rapid, shallow breathing) and there is a small increase in TP. This is called the first phase of pulmonary irritation (P1).

F: During pulmonary irritation

Note the major changes: the rapid shallow breathing increases and the duration of TP increases to an apneic pause between breaths. TP will continue to increase and will result in a net decrease in f. This is called pulmonary irritation (P).

COMPUTERIZED METHOD FOR SI,A,&P

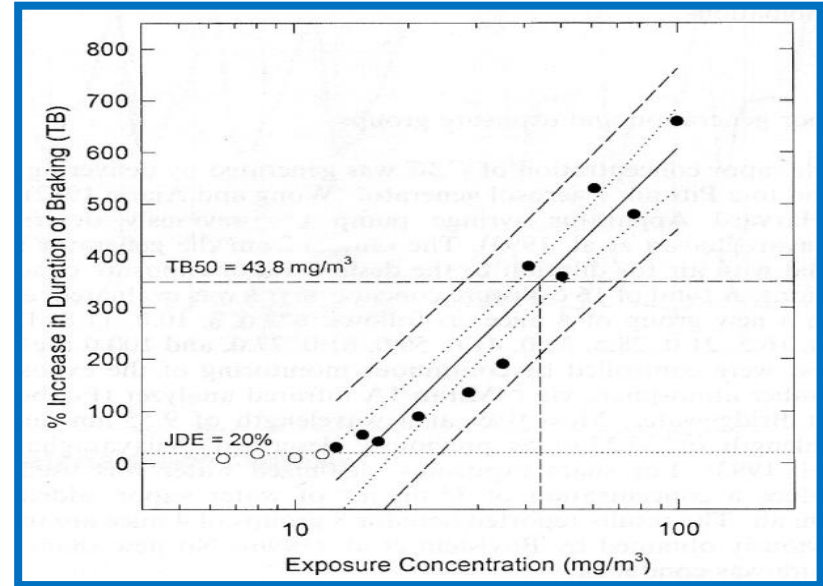
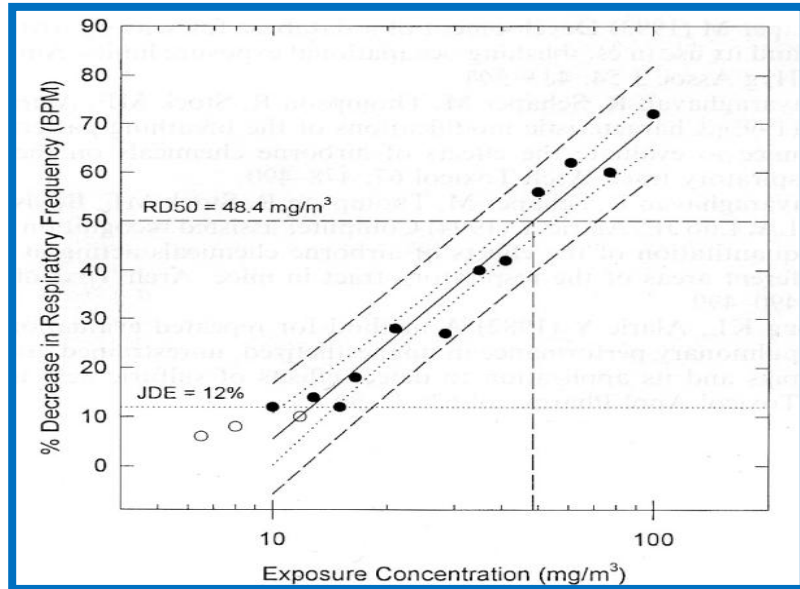
SENSORY IRRITATION: MEASURING POTENCY

Given the above, we can assess whether or not an airborne chemical has sensory irritating properties by measuring TB or by simply measuring respiratory frequency (f or BPM) provided that the decrease in f is due to an increase in TB, obviously.

COMPUTERIZED METHOD FOR SI,A,&P

The figures presented on the next slide*: Concentration-response relationship for %BPM and %TB, during exposure to CBC. The regression line (*solid*), 95% CI (*dotted lines*), and 95% PI (*dashed lines*) are presented. *Open circles* indicate data points omitted, see text.

*From: Alarie, Y. (1998). Computer-based bioassay for evaluation of sensory irritation of airborne chemicals and its limit of detection. Arch. Toxicol. 72: 277-282.



$$\% \text{BPM} = 59.51 + (64.99 \times \log \text{ concentration}), r^2 = 0.96$$

$$\% \text{TB} = 824.9 + (715 \times \log \text{ concentration}), r^2 = 0.93$$

BRIEF UPDATE OF RESULTS FROM 1966-2007

- Continued progress was made, increasing the number of chemicals evaluated and a review with QSAR was published by Y. Alarie in 1973 (Crit. Rev. Toxicol. 2: 299-363)

- M. Schaper published in 1993 a large database (total of 244 chemicals), containing 89 VOCs for which RD50 and TLV values were available, again with very good linear regression relationships found (Am. Ind. Hyg. Assoc. J. 54: 488-544).

- Using this database, Alarie et al. (1998 Arch. Toxicol. 72: 125-140) extracted 145 VOCs, 83 reactive and 59 non-reactive and were able using Abraham's chemical descriptors for nrVOCs to estimate their potency. Excellent results were obtained, but restricted only to nrVOCs. Results for rVOCs were explained separately.

-
- Original and computerized methods were used by investigators in China, Denmark, Finland, France, Germany, India, Japan, Poland, etc. More chemicals have been added, or the same chemicals have been tested by several laboratories.

-
- Therefore, the 1993 database of Schaper
 - has now been updated and contains 102 chemicals for which we also have TLVs. Since several chemicals have also been evaluated by several laboratories, we now have a total of 184 entries.

UPDATED TABLE: TABLE 1

The updated 1993 Schaper table contains the following:

- Chemical # and number of entries for each chemical
- CAS number and TLV Documentation date
- Type of mice used for exposure
- RD50 value obtained (ppm)
- RD50 x 0.03 (ppm)
- 1991-1992 TLV (ppm)
- 2015 TLV (ppm)
- 2015 Basis (health effects) for TLV

***TOTAL NOW:
102 CHEMICALS
184 ENTRIES***

<u>EXAMPLES</u>	CAS #	Type of Mice Used	RD ₅₀ ppm	RD ₅₀ x 0.03 ppm	1991-1992 TLV ppm	2015 TLV ppm	2015 TLV Basis
Chemical							
Acetone <chem>(CH3)2C=O</chem>	67-64-1 (1996)	OF1 SW	23480 77516	704 2325	750	500	URT & eye irritation; CNS impairment; hematologic effect
Acrolein <chem>CH2=CHCH=O</chem>	107-02-8 (1995)	SW B6C3F1 BALB/C SW SW CF1 C57B1	1.03 1.41 1.66 1.68 2.70 2.90 1.59	0.031 0.042 0.050 0.050 0.081 0.087 0.048	0.1	C0.1	URT & eye irritation; pulmonary edema; pulmonary emphysema

<u>EXAMPLES</u>	CAS #	Type of Mice Used	RD50 ppm	RD50 x 0.03 ppm	1991-1992 TLV Ppm	2015 TLV ppm	2015 TLV Basis
Chemical							
Allyl Alcohol CH₂=CH-CH₂OH	107-18-6 (1996)	OF1 ICR CF1	1.6 2.5 3.9	0.048 0.075 0.117	2.0	0.5	URT & eye irritation
n-Propyl Alcohol (n-Propanol) CH₃-CH₂-CH₂OH	71-23-8 (2006)	OF1 SW CF1	4780 12704 13660	143 381 409	200	100	URT & eye irritation

TABLE 1 - DISCUSSION POINTS

ACETONE: nrVOC, low potency, factor of 3 between the two types of mice, drop in TLV 1991 to 2015, added effect to URT

ACROLEIN: rVOC, very high potency, factor of 3 between different types of mice, added C in 2015, added effects to URT

ALLYL ALCOHOL: rVOC, very high potency, factor of 3 between different types of mice, drop in TLV from 1991 to 2015

n-PROPYL ALCOHOL: nrVOC, very low potency, shows effect of unsaturation in allyl alcohol, again a factor of 3 between different types of mice, again drop in TLV from 1991 to 2015

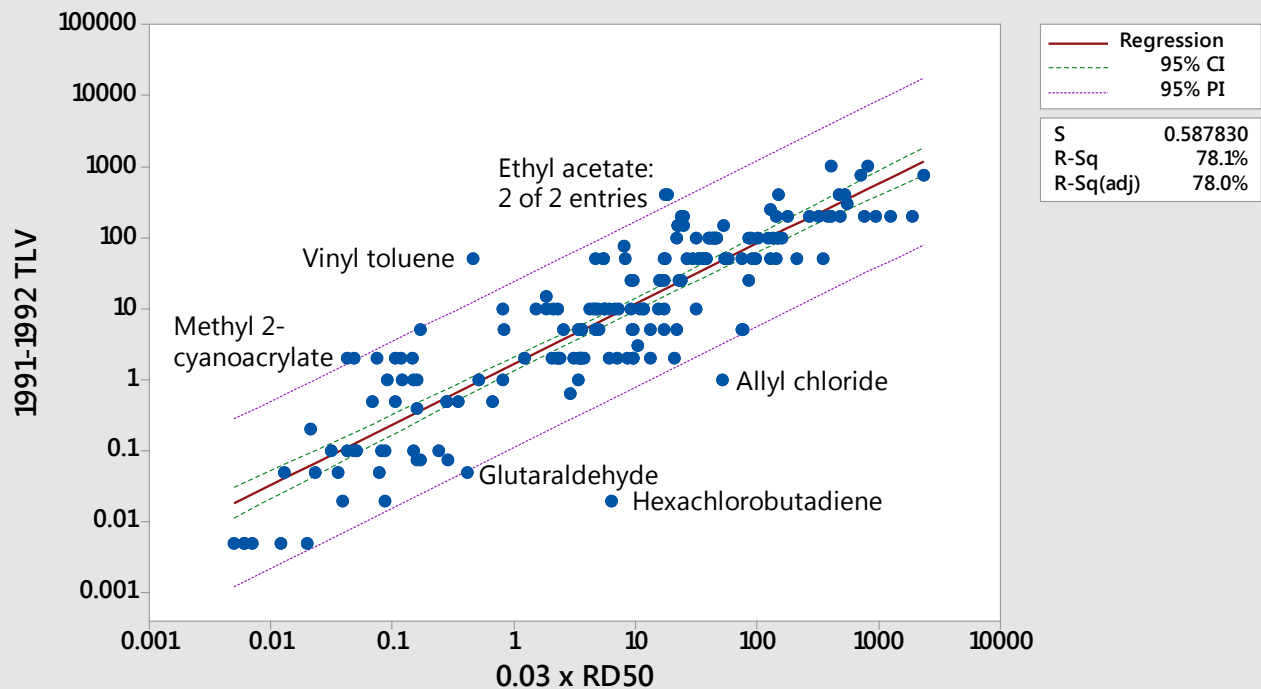
TABLE 1 - SUMMARY

Chemical	AVG RD50 x 0.03 (ppm)	1991-1992 TLV (ppm)	2015 TLV (ppm)
Acetone	1515	750	500
Acrolein	0.056	0.1	C0.1
Allyl Alcohol	0.08	2.0	0.5
n-Propyl Alcohol	311	200	100

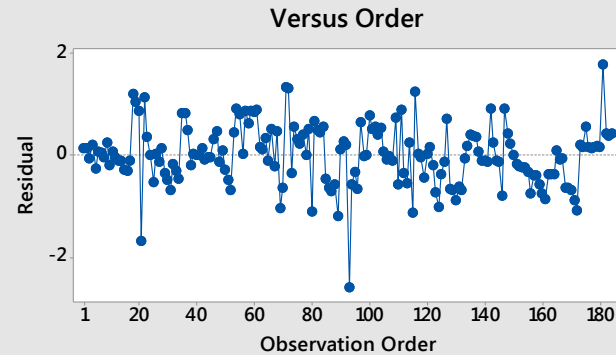
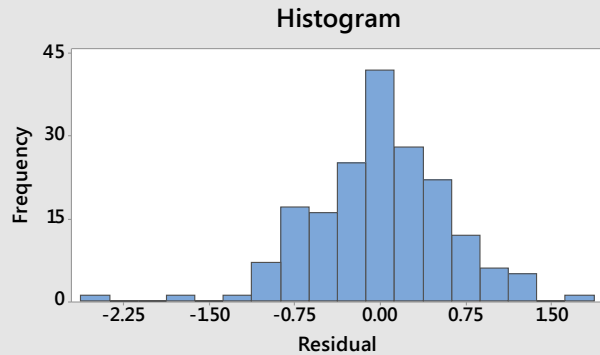
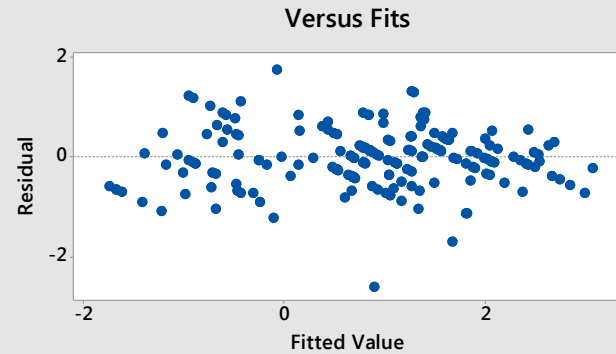
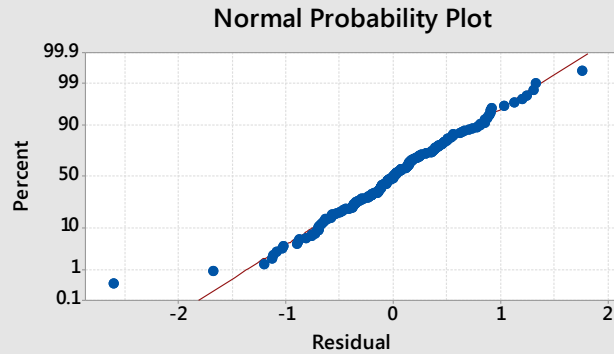
The entire table is available at www.pitt.edu/~rd50, and is also available at this lecture site.

1991-1992 TLV versus 0.03 x RD50 for 184 entries from 102 chemicals

$$\log_{10}(\text{1991-1992 TLV}) = 0.2186 + 0.8486 \log_{10}(0.03 \times \text{RD50})$$



Residual Plots for $\log_{10}(1991-1992 \text{ TLV})$

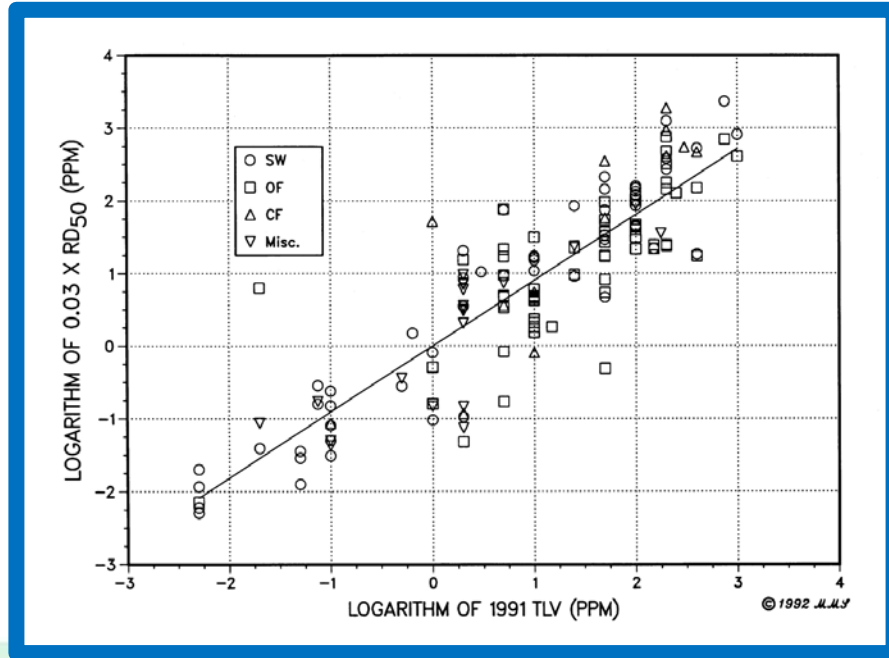


REGRESSION ANALYSIS

$0.03 \times RD_{50}$ VS 1991-1992 TLV

Plot includes all strains of mice used to obtain RD_{50} values

$R^2 = 0.78$

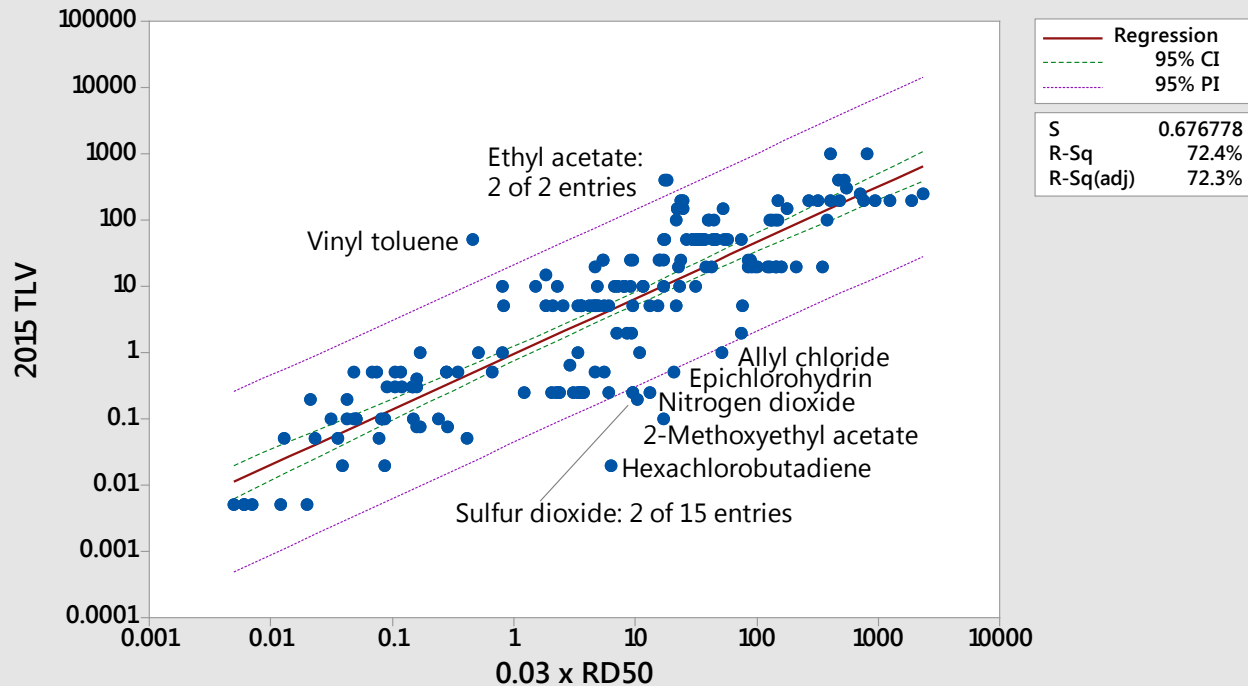


Using the data from Table 1, the next two slides illustrate:

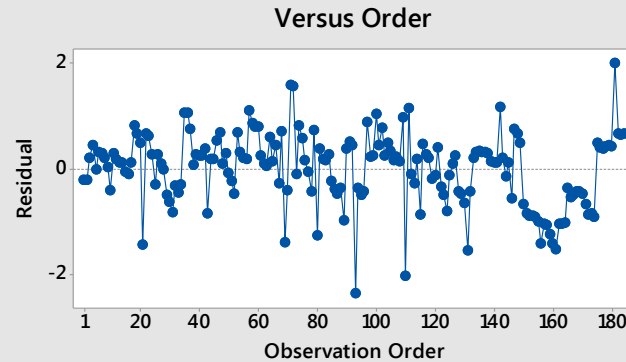
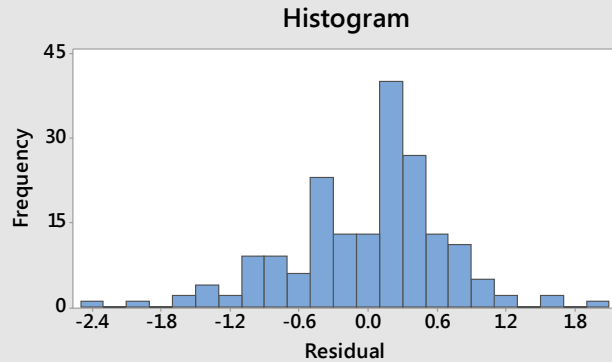
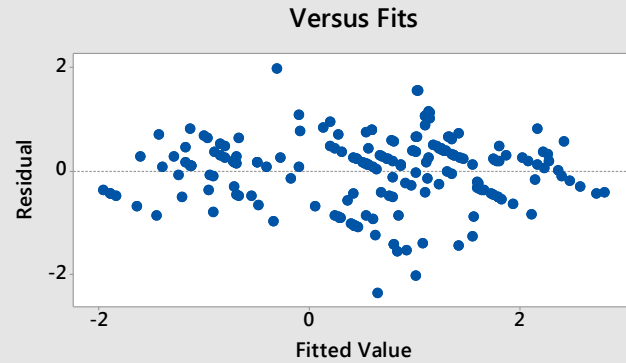
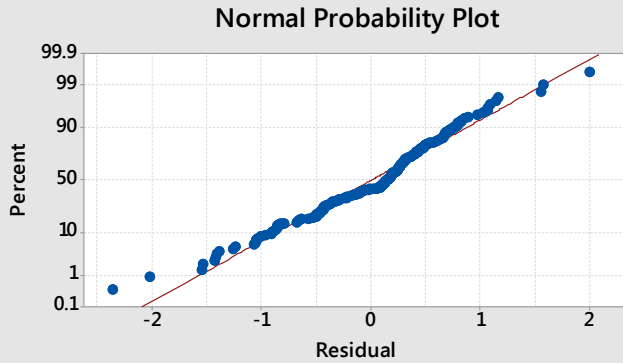
1. Linear regression analysis of 2015 TLV versus $0.03 \times RD_{50}$
2. Analysis of the residuals for the least squares linear regression analysis of 2015 TLV vs. $0.03 \times RD_{50}$ in the figure can be inspected and shows that the linear model is adequate. Outliers are easily identified as noted on the linear regression analysis plot. Statistical analysis was conducted using Minitab 17. In the “Versus Order” plot, the observation numbers correspond to the entry numbers in Table 1.

2015 TLV versus 0.03 x RD50 for 184 entries from 102 chemicals

$$\log_{10}(\text{2015 TLV}) = -0.01900 + 0.8392 \log_{10}(0.03 \times \text{RD50})$$



Residual Plots for log₁₀(2015 TLV)



MORE RECENT APPROACHES

- Luan, F. et al. (2006) Chemosphere 63: 1142-1153
These investigators were also able to get excellent results for nrVOCs, using chemical descriptors and using the 1993 database from Schaper. Some progress was made for the rVOCs.
- Gupta, S. et al. (2015). Ecotoxicology 24: 873-886.
Estimating irritation potency of volatile organic chemicals using QSARs based on decision tree methods for regulatory purpose. Again, the database from Schaper was used.

- The importance of the approach of Gupta et al. (2015) is that it can take both nrVOC and rVOCs and will now be presented here in the slides below.
- Another very recent article is authored by Abraham, M.H. et al. (2016). An assessment of air quality reflecting the chemosensory irritation impact of mixtures of volatile organic compounds. *Environ. International* 86: 84-91.

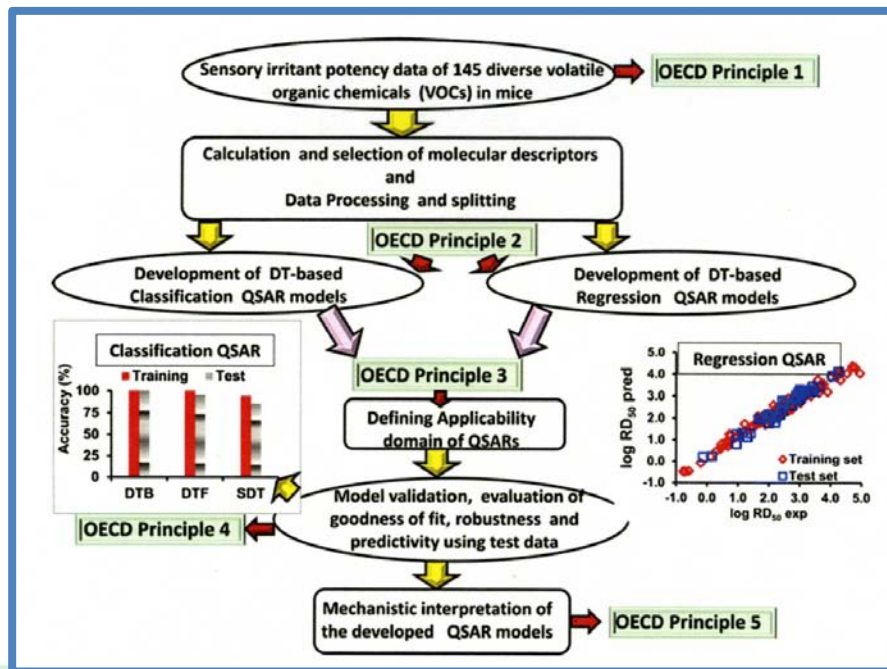
Gupta et al. (2015) are following 3 important steps, well-described in their article. They have used 145 VOCs, the same as used by Alarie et al. (1998) above, and all are from the 1993 Schaper database.

- Use the QSAR modeling steps prescribed by the OECD
- Calculated molecular descriptors for the both nrVOCs and rVOCs using known methods
- Using decision tree classification techniques to optimize the results

The figures below are from the Gupta et al. article.

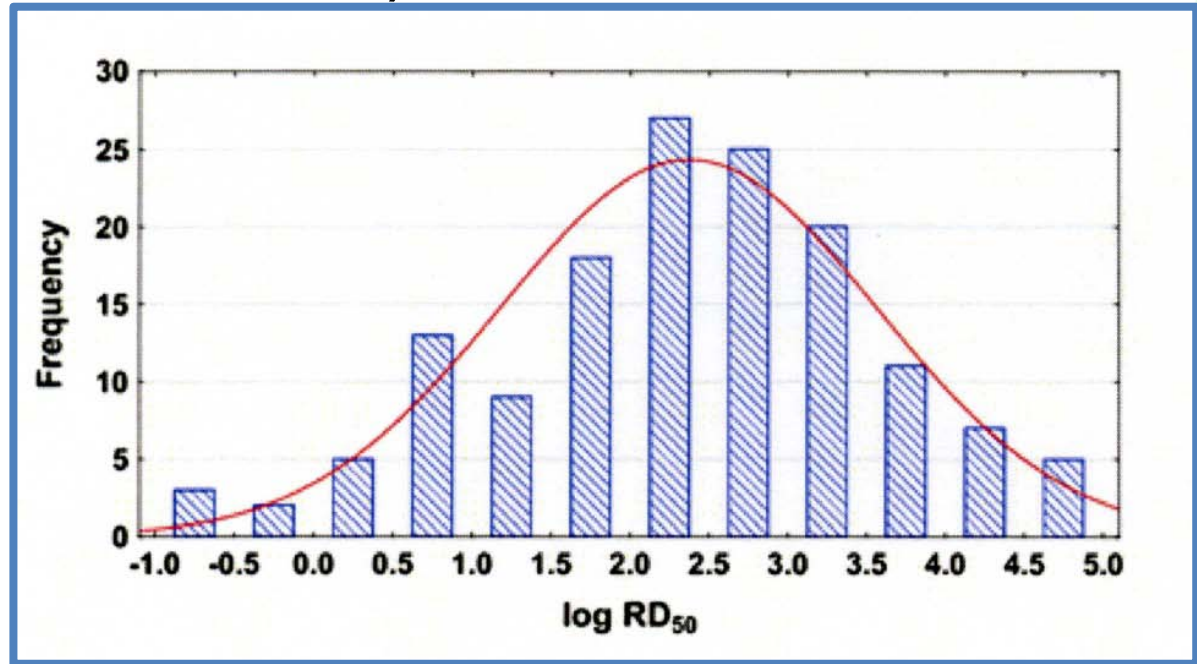
QSAR MODELING STEPS AND RESPECTIVE OECD PRINCIPLES

FROM: GUPTA
et. al., 2015



HISTOGRAM OF SENSORY IRRITATION POTENCY (LOG RD50) OF VOCs IN MICE

FROM: GUPTA
et. al., 2015



MEASURED VS. PREDICTED LOG RD₅₀

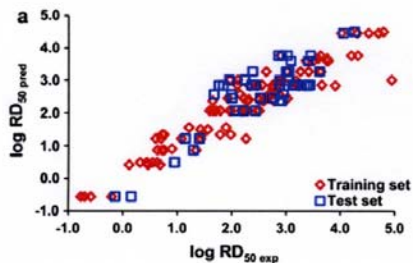
Measured vs. model predicted
log RD₅₀ values for training
and test sets in:

a) SDT (Single Decision Tree)

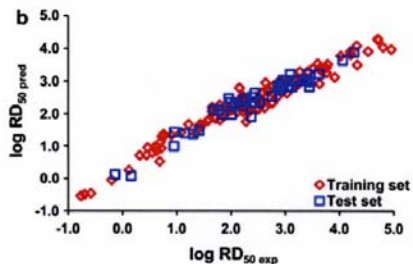
b) DTF (Decision Tree Forest)

c) DTB (Decision Tree Boost)

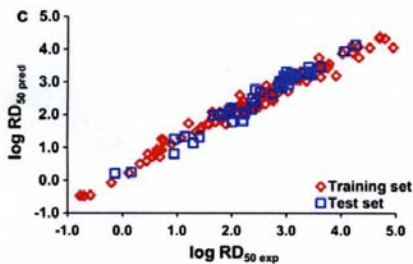
a)



b)



c)



GUPTA ET AL. (2015) YIELDED
GOOD RESULTS, BUT THIS IS FOR RD₅₀S.

WE NEED TLVs.

- How do we get to TLVs?
- We already know there was a good correlation between 0.03 x RD₅₀s and TLVs in 1980 and 1993.
- Is it still good for the 2015 updated database on RD₅₀s and the 2015 TLVs?
- The answer is **YES** as presented in the regression analysis graphs on the previous slides.

NEED TO TEST ABOVE QSAR WITH NEW CHEMICALS

- The above QSAR contains 145 chemicals, but only 102 have a TLV. This is not a problem.
- However, we need to test how good the potency estimates would be for new chemicals. This is required for any QSAR.

- A total of 114 chemicals (all nr or r VOCs) were identified with the basis for their TLV being 'URT irr', 'Eye irr' in the 2015 ACGIH TLV Booklet. These are presented in the table below.
- We could obtain RD_{50} values in mice to verify how close the estimated TLV ($0.03 \times RD_{50}$) would be. This is not necessary.
- Since $TLV = 0.03 \times RD_{50}$, it follows that $RD_{50} = TLV / 0.03$.
- We would now have a total of 216 chemicals.

TABLE 2. LIST OF CHEMICALS WITH THRESHOLD LIMIT VALUES (TLVs) PRIMARILY BASED ON SENSORY

Chemicals	CAS #	Documentation Date ¹	TLV-TWA or STEL ² (ppm)	TLV Basis ³
Acetic anhydride	108-24-7	2010	10	URT & eye irr; pulm func

No RD_{50} values are available for these chemicals.

This table includes only volatile organic chemicals (VOCs).

CHEMICALS BEGINNING WITH “A”

Acetic anhydride	108-24-7	2010	10	URT & eye irr; Pulm func
Acetophenone	98-86-2	2008	10	URT irr; CNS impair, Pregnancy lost
Acrylic acid	79-10-7	1986	2	URT irr
Adiponitrile	111-69-3	1990	2	URT & LRT irr
Allyl bromide	106-95-6	2011	0.1	Eye & URT irr
Allyl propyl disulfide	2179-59-1	2001	0.5	URT & eye irr

CHEMICALS BEGINNING WITH “B”

Benzotrichloride	98-07-7	1994	0.1	Eye, skin & URT irr
Benzoyl chloride	98-88-4	1992	0.5	URT & eye irr
Benzyl acetate	140-11-4	1990	10	URT irr
sec-Butanol	78-92-2	2001	100	URT irr; CNS impair
iso-Butene	115-11-7	2007	250	URT irr; body weight eff
sec-Butyl acetate	105-46-4	1965	200	Eye & URT irr
n-Butyl acrylate	141-32-2	1996	2	Irr
n-Butyl mercaptan	109-79-5	1968	0.5	URT irr
o-sec-Butylphenol	89-72-5	1977	5	URT, eye & skin irr

WHAT TO EXPECT IF USING THE QSAR WITH THESE ADDITIONAL CHEMICALS

Looking at the list of chemicals in the table on the previous slides, an organic chemist would reach the conclusion that we would expect several outliers. This is simply because many of them would fall outside of the chemical domain in the current QSAR.

However, this is not a problem. If so, new and expanded training and test sets would be appropriate for again testing how valuable a QSAR could be to evaluate the TLV of yet untested chemicals, provided that they are VOCs.

We can also expect new chemicals to be introduced in the work place. The computerized method has greatly expanded our ability to quickly establish the likely location/s of the respiratory tract that may be most affected during inhalation exposures.

ACKNOWLEDGEMENT

Over the years, much progress was made with the collaboration of Michael H. Abraham, University College London, Gunnar D. Nielsen, National Institute of Occupational Health, Denmark and Michelle M. Schaper, U.S. Department of Labor, as well as many students, postdoctoral fellows, and visiting scientists.



Questions or Comments may be sent to: rd50@pitt.edu

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