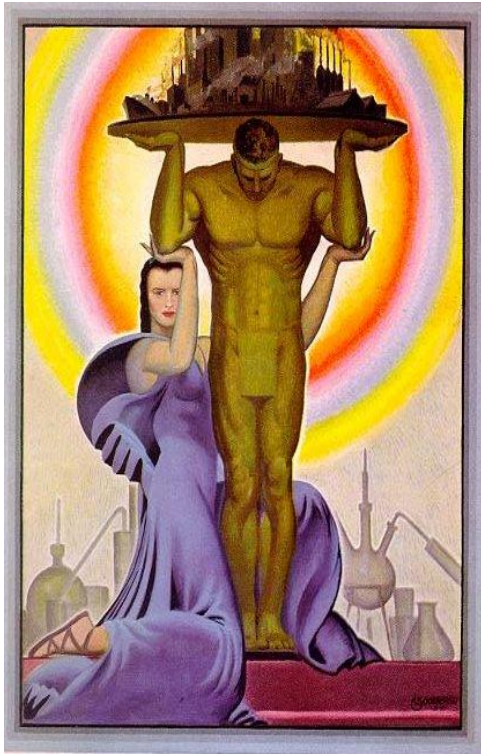


Ancient Cellular Toxic Defense Meets A New World Of Chemicals

David Epel, Hopkins Marine Station of
Stanford University



Optimism of the early 20th century.....gives way to cynicism



"Better things for better living.....through chemistry".



My goal ... show a way to avoid these problems

- Review how organisms handle toxicants
- Some chemicals evade this defense
- Ancient and recent stories
- A paradigm that predicts.....
- Problems with the paradigm
- Some solutions

We live in a chemical world



....some are natural

....some are synthetic



Two approaches to handle toxic chemicals



Liver—chemical approach...
modifying....less toxic



Epithelial layers—bouncer
approach...keeping out of cell in
first place

The problem...some chemicals evade these protective mechanisms

- Some are toxic
- Some accumulate and concentrations so high that become toxic

Lessons learned from history...

- Get used to the desirable features
- Consumer complacency
- Industry fights regulation
- ...and years later....here we are

A ROMAN POEM ABOUT LEAD

Hence gout and stone afflict the human race;
Hence lazy jaundice with her saffron face;
Palsy, with shaking head and tott'ring knees.
And bloated dropsy, the staunch sot's disease;
Consumption, pale, with keen but hollow eye,
And sharpened feature, shew'd that death was nigh
The feeble offspring curse their crazy sires,
And, tainted from his birth, the youth expires.

*Description of lead poisoning by an anonymous
Roman hermit; translated by Humelbergius
Secundus, 1829*

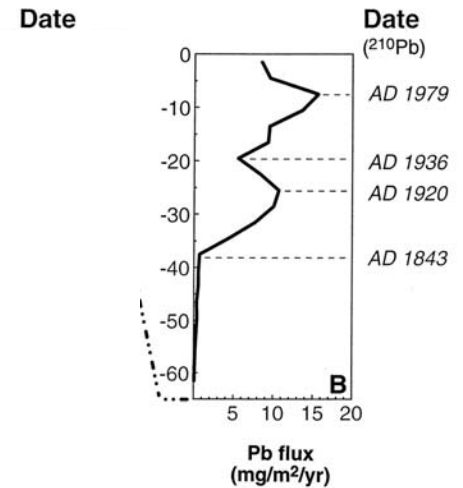
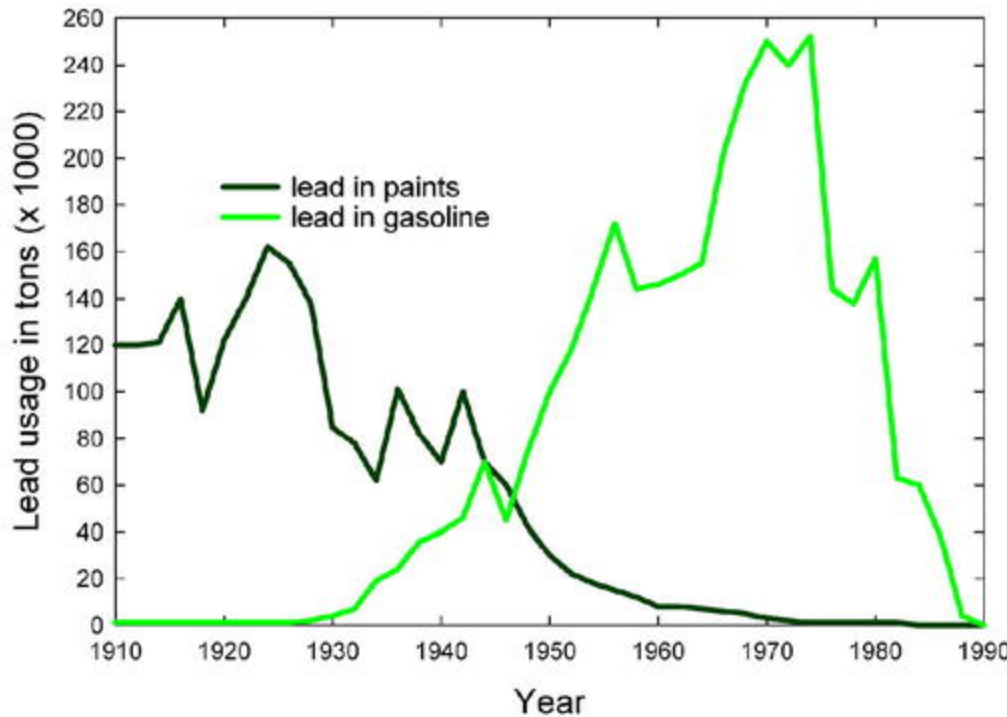
**USES OF LEAD—FACE POWDER, MASCARA,
PLUMBING (PLUMBOUS), WINE STORAGE, ETC. ETC.**

Our contemporary love affair with lead

DISCOVERY OF TETRAETHYL LEAD FOR GASOLINE 1921—THE ETHYL CORPORATION

- Problem—15 deaths of workers in the Ethyl Corporation plant
- 1925-Surgeon General report decides no problem if use "controlled by proper regulations"
- Calls for removal of lead opposed by industry for decades

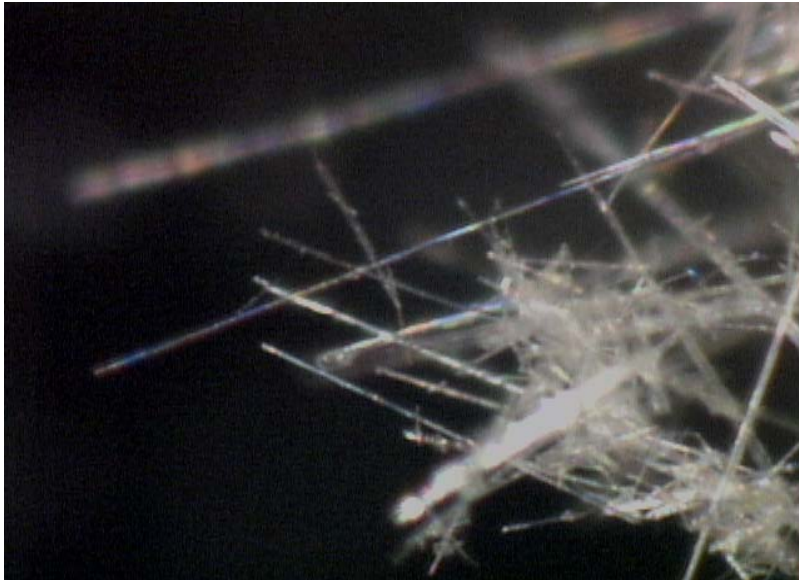
- By 1975-annual lead production in US was 5200 gms/person (in Roman times, was 500 gms/person)
- 1976-lead in gas finally prohibited



History sadly repeats itself....

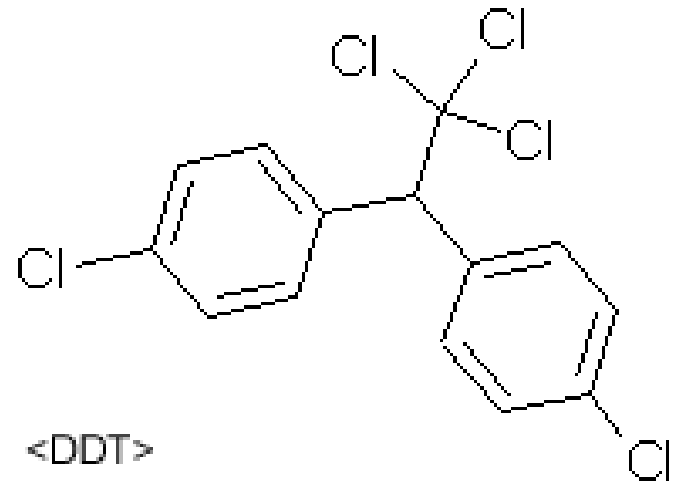
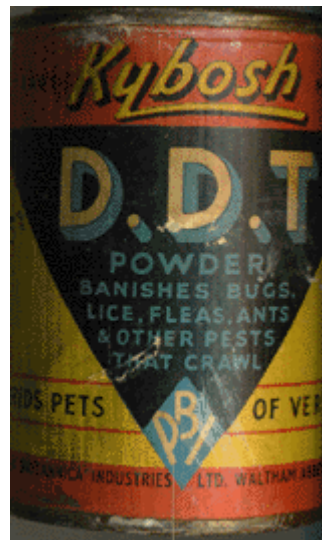
- **Romans knew about lead poisoning—**
- an EPA report in 1985 said".....they were so fond of its diverse uses that they minimized the hazards it posed. Romans of yesteryear, like Americans of today, equated limited exposure to lead with limited risk."
- **We also knew lead was a problem ...took 50 years to fix**

USE OF ASBESTOS



DISCOVERY OF DDT

- Paul Muller, Nobel laureate, 1948



DDT helped to control malaria and other insect-borne diseases



FIGURE 41.—DDT residual spraying with power spray.

...from the introduction of Paul Muller on the awarding of his Nobel Prize in Medicine

- ..."A short story perhaps illustrates better than many words how the substance acts. In 1945, when DDT was still relatively untried, I met an English Major in Germany who told me he had treated the window pane of his room with DDT since he was plagued by masses of flies. After the DDT solution had been sprayed on, the flies died and lay in heaps on the window ledge. The following morning a German soldier entered and thoroughly cleaned the window. When the Major noticed this he couldn't help crying «Goodbye my DDT!». But this farewell was uncalled for. In spite of the thorough cleaning, the window pane retained its deadly action on the flies. This little story amply illustrates how **persistent DDT is and how small the dosage required....**"

The profligate use of DDT creates problems



Special Collections Department, Pullen Library, Georgia State University

DDT turns out to be a global pollutant

1098

NATURE, VOL. 220, DECEMBER 14, 1968

Polychlorinated Biphenyls in the Global Ecosystem

by

R. W. RISEBROUGH
P. RIECHE

Institute of Marine Resources,
University of California, Berkeley 94720

D. B. PEAKALL

Division of Ecology and Systematics,
Cornell University, Ithaca,
New York 14750

S. G. HERMAN

Department of Zoology,
University of California, Davis 95616

M. N. KIRVEN

San Diego Natural History Museum,
PO Box 1390, San Diego, California 92112

Polychlorinated biphenyls are widely dispersed in the global ecosystem, and are powerful inducers of hepatic enzymes which degrade oestradiol. Together with other chlorinated biocides, such as DDT, they could account for a large part of the aberration in calcium metabolism which has been observed in many species of birds since the Second World War.

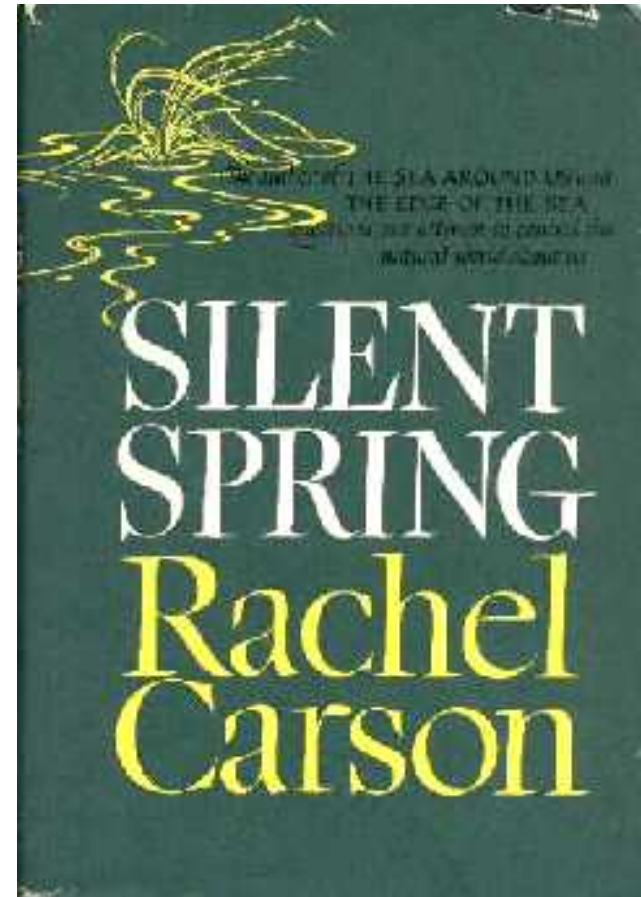
DECLINING populations of raptorial and fish-eating birds in Great Britain¹ and North America² have produced thin-shelled eggs since the period after the Second World War. A widespread change in the chemical environment which affected the calcium physiology of these species evidently occurred at that time. The chlorinated hydrocarbons, which came into general use in the 1940s, may now be the most abundant synthetic pollutants present in the global environment³. Thin eggshells have been found only in species which accumulate high concentrations of these compounds: relatively uncontaminated populations of these species continue to produce normal eggs^{1,2}.

Calcium metabolism in birds is intimately related to reproductive metabolism and is to a large extent regulated by steroids such as oestrogen and vitamin D⁴. The deposition of medullary bone, the chief source of calcium during egg and eggshell formation, is controlled by the steroid sex hormones⁴⁻⁷, and hens deficient in vitamin D

2,2-bis(*p*-chlorophenyl)ethane), DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane) and toxaphene are dehydrochlorinated by saponification with alcoholic KOH. PCB is not degraded by either procedure.

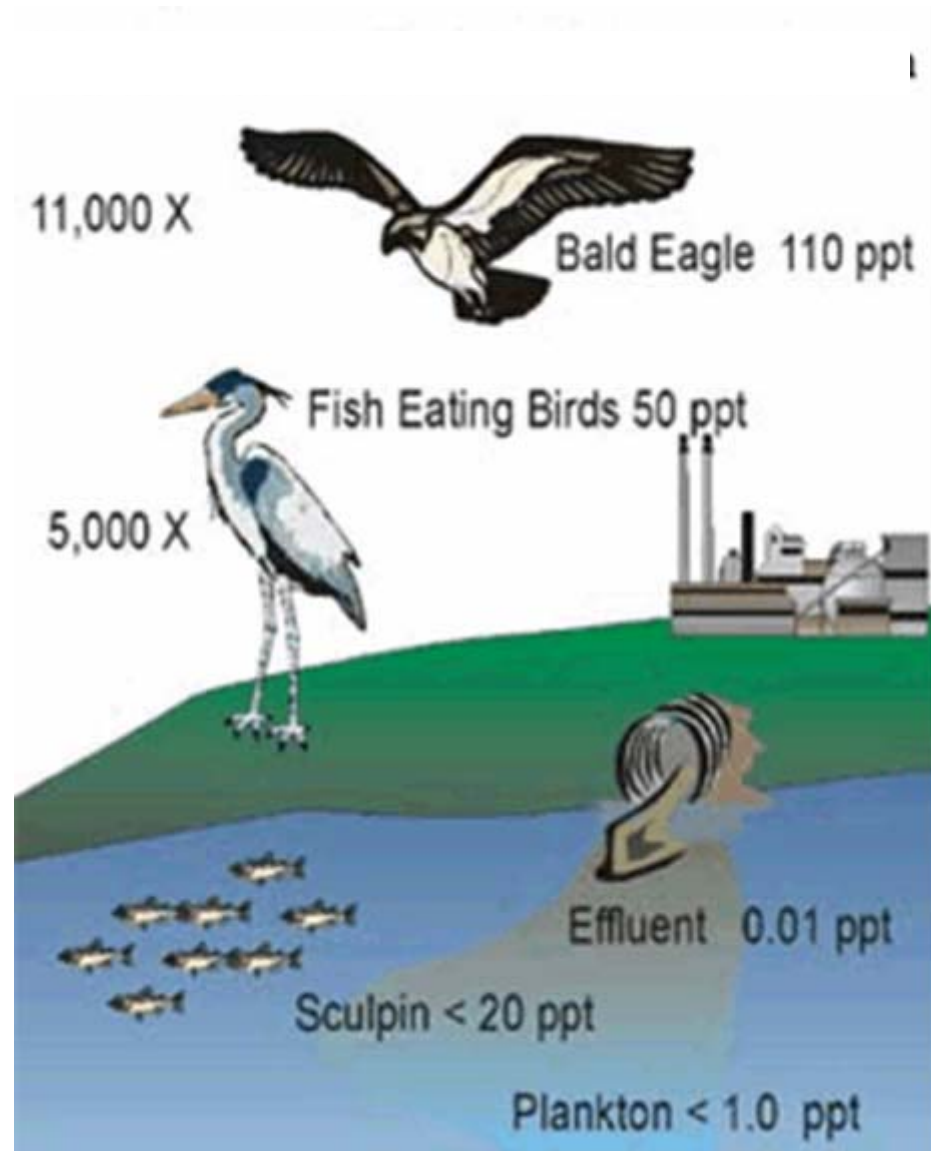
Table 1. CHLORINATED HYDROCARBONS IN NORTH AMERICAN PEREGRINE FALCONS

Sample	Dieldrin	Total DDT*	Per-centage DDE	PCB	DDT/PCB
1. Unhatched egg †					
Baja California (wet)	0.11	102	97	10.2	10
2. Second year ‡ migrant from Arctic					
Breast muscle (wet)	NM	99	94	28	3.5
Breast muscle (dry)		296		84	
Brain (wet)	NM	85	98	21	4.7
Carcass (wet)	0.87	70	93	19.7	3.5
Carcass (lipid)	62.5	5,000		1,420	
3. Immature, California §					
Breast muscle (wet)	NM	14.4	90	9.4	1.5
Liver (wet)	NM	7.7	92	4.5	1.7
Brain (wet)	0.04	2.8	89	1.5	1.9
Brain (lipid)	0.50	35		19.3	
Carcass (wet)	0.11	20.2	92	10.8	1.9
Carcass (lipid)	1.6	200		120	

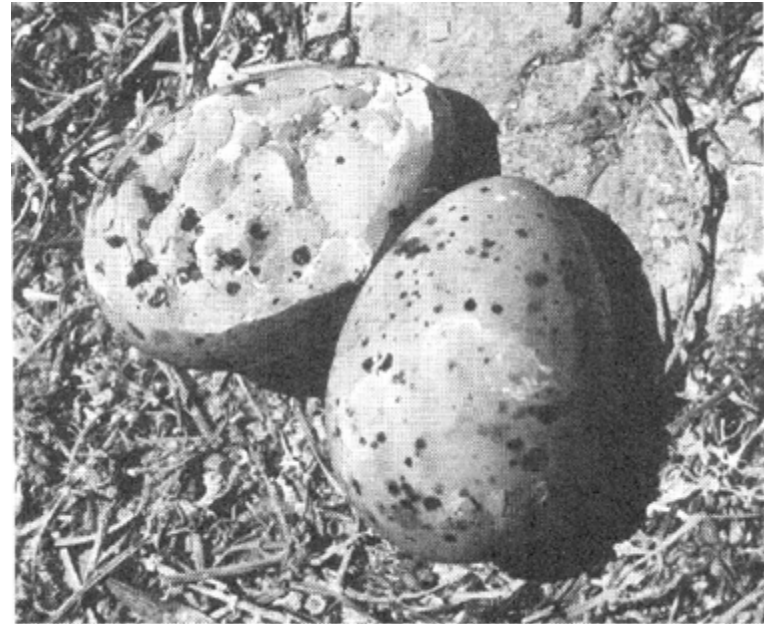


A reviewer for the *Christian Science Monitor*, writing about *Silent Spring* said, "Miss Carson has undeniably sketched a one-sided picture. But her distortion is akin to that of the painter who exaggerates to focus attention on essentials. It is not the half-truth of the propagandist."

How does DDT accumulate in the food chain?



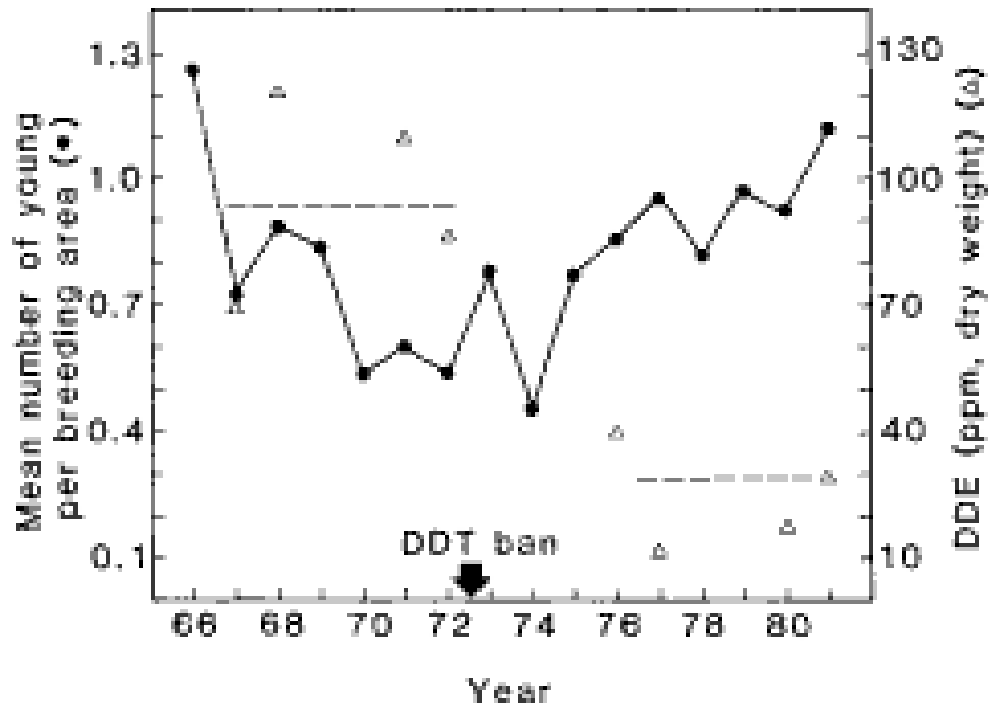
DDT levels got as high as milligrams in raptor eggs



Crushed and normal eggs in a Caspian tern nest. DDT residues and consequent thinning have been related to such breakage.

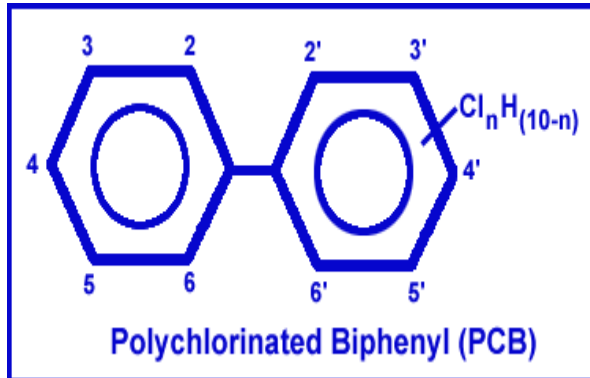
- Consequences...
- Eggs did not survive

Recovery of bald eagle populations after ban of DDT

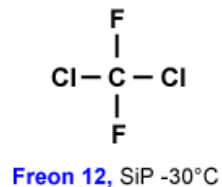
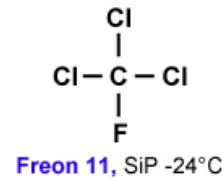
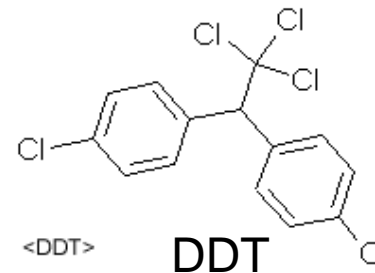


DDT, PCB's & CFC's As Global Pollutants----

How Could This Happen?



PCB



FREON

Periodic table...an index of relatedness among chemicals

	I	II	III	IV	V	VI	VII	VIII
1	1 Hydrogen H 1							2 Helium He 4
2	3 Lithium Li 7	4 Beryllium Be 9	5 Boron B 11	6 Carbon C 12	7 Nitrogen N 14	8 Oxygen O 16	9 Fluorine F 19	10 Neon Ne 20
3	11 Sodium Na 23	12 Magnesium Mg 24	13 Aluminum Al 27	14 Silicon Si 28	15 Phosphorus P 31	16 Sulfur S 32	17 Chlorine Cl 35	18 Argon Ar 40
4	19 Potassium K 40	20 Calcium Ca 40	(plus others in here)				35 Bromine Br 80	(etc.)

The DDT and PCB paradigm

- Fat-soluble and persistent chemicals will accumulate in living tissue, animals as well as humans.
- If produced in large enough amounts, will pollute the globe and could have disastrous effects

THIS PARADIGM CAN WORK— PBDE'S ARE BROMINATED CHEMICALS ARE FAT SOLUBLE

- August 10, 2003, Sunday
California to Ban Chemicals Used as Flame Retardants

By JENNIFER 8. LEE (NYT) 872 words
Late Edition - Final , Section 1 , Page 14 , Column 5
- ABSTRACT - Gov Gray Davis signs legislation that will eventually ban flame retardants known as PBDE's in California, citing research showing rapid accumulation of PBDE's in humans and wildlife; scientists says PBDE's may be among chemicals that harm health at levels that accumulate in body; ban will start in 2008, compromise date set by chemical industry and state legislators; PBDE's are found in plastics and foams, and they have become widely used as governments have raised flammability resistance requirements for products; European Union has imposed similar ban (M)

BUT RECENT WORK SHOWS THE PARADIGM DOESN'T ALWAYS WORK



Current concern—"emerging" toxicants in the environment.

New chemicals from industrial processes with global effects



1950's—1970's —DDT, PCB's and CFC's.

2000—flame retardants, PFCs, herbicides, plasticizers and personal care products and pharmaceuticals.

Perfluoro's in marine mammals

TABLE 1. Ranges of Concentrations of PFOS (ng/g, wet wt, for Liver and Muscle Tissues and ng/mL for Blood Plasma) in Wildlife. Mean Concentrations Are Given in Parentheses

species	location	tissue	n	PFOS
Aquatic Mammals				
ringed seal	Canadian Arctic	plasma	24	<3–12
ringed seal	Baltic Sea	plasma	18	16–230 (110)
ringed seal	Norwegian Arctic	plasma	18	5–14 (9)
gray seal	Baltic Sea	plasma	26	14–76 (37)
gray seal	Canadian Arctic	plasma	12	11–49 (28)
California sea lion	Coastal California	liver	6	<35–49
elephant seal	Coastal California	liver	5	<35
harbor seal	Coastal California	liver	3	<35–57
river otter	Northwestern USA	liver	5	34–990 (330)
sea otter	Coastal California	liver	8	<35
northern furseal	Coastal waters of Alaska	liver	14	<35–120
polar bear	Alaska, USA	liver	17	180–680 (350)
mink	Midwestern USA	liver	18	970–3680 (2630)
bottlenose dolphin	Mediterranean Sea	liver	5	170–430 (270)
striped dolphin	Mediterranean Sea	liver	4	65–160 (100)
weddel Seal	Terra Nova Bay, Antarctica	liver	1	<35
Ganges river dolphin	Ganges River, India	liver	2	<35–81

These cellular defenses are not working



Liver—chemical approach...modifying the chemical so less toxic



Stomach (placenta, blood-brain barrier, blood-testis barrier)
—**barricade or bouncer** approach...keeping the chemical out of cell in first place

Why global? detox mechanisms can't act...or act slowly... on some chemicals

Examples of global chemicals

- Nitro-aromatics (musk fragrances)
- Halogenated organics as PBDE, DDT AND PCB
- Perfluorocarbons

Hypothesis to explain global occurrence

- Free entry to cell, as efflux transporters don't recognize
- Can't be metabolized
- Can't be bounced out of cells
- So remains as legacy of past exposure

Our directions

- Fat solubility doesn't always work
- Can inability to bounce out chemicals be used to predict global chemicals?
- Regulatory or policy value?

Topsy-turvy or upside down thinking....



- Don't focus on what the detox can do....
 - Focus on what it can't handle...what is not recognized...

If not recognized by the
bouncers....free entry into cell

- Short term --thalidomide
- Long-term --global pollutants

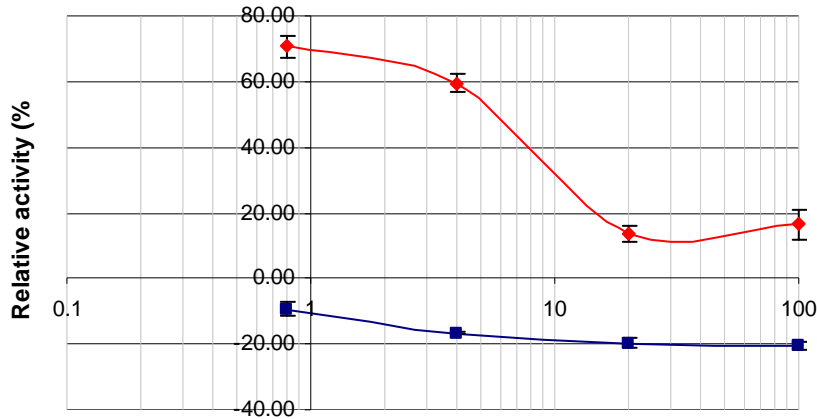
Determining if global chemicals are recognized...are substrates?

- Substrate? Measure effect of chemical on activity of transport molecule ...if a substrate the activity will increase.
- Inhibitor? Measure effect of chemical on the stimulation of activity by a **known substrate**...if an inhibitor the activity will decrease.
 - These studies done in collaboration with Solvo Biotechnology in Hungary

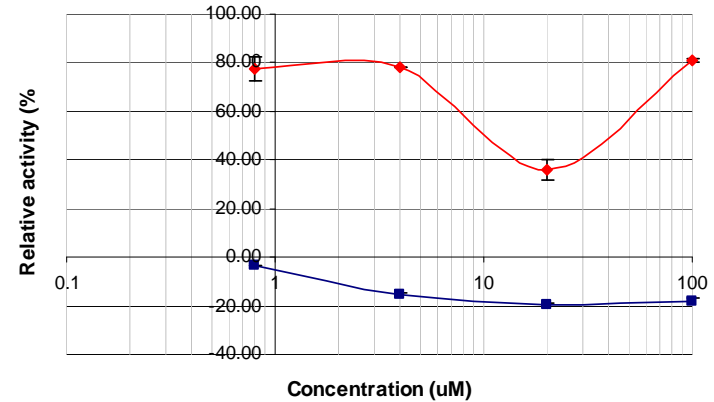
Results of test....the "globals" are not substrates....At high concentrations they inhibit the transporter activity

ATPase stimulation
ATPase inhibition-index of whether substrate

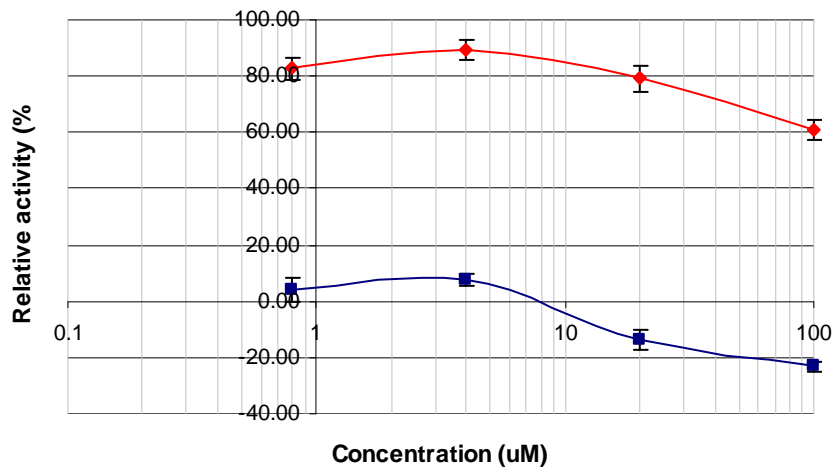
57 - Aroclor 1254



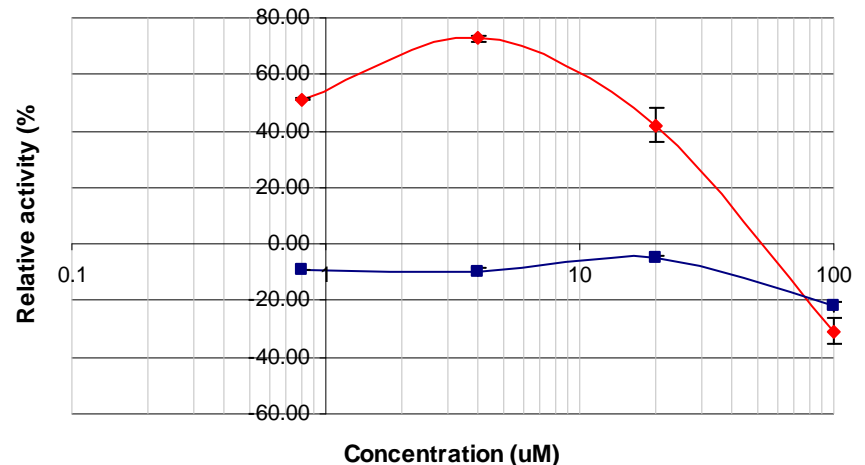
58 - DDT



60 - Musk xylene



62 - PFNA



Conclusions

- So called global pollutants are not seen by the bouncers...so freely enter and can't be bounced out
- Indeed, any drug not recognized by the transporter can be a problem....
 - Thalidomide as example

Another way of stating these results

The reason chemicals become global pollutants is that

1. Are permeable
2. Are not metabolized
3. Cannot be removed by efflux transporters..the bouncers or garbage collectors of cells



How does this relate to retardants?

...the chemical industry makes consumer protection look like a game of whack-a-mole.

– Donald Kennedy

Stuart Carlson



QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

We can't do this in a piecemeal fashion

- Need some general guidelines
- ...against global pollutants
- ...against so called endocrine disruptors

One proposal—Regulation by an Environmental FDA...an ECA

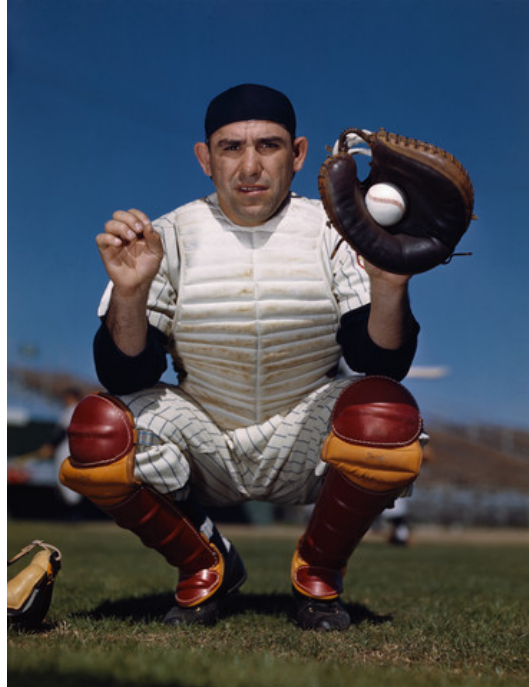
- Determine if permeable...Kow is indicator
- Determine if "bouncer"/transporter can recognize them
- Determine if can be metabolized
- If large amounts manufactured, will accumulate in organisms...become global pollutants

- Either don't allow to be made or only make small amounts
- If they have potential to be global pollutants, don't even think about making them in bulk
- This is same principle used by drug companies in screening drugs—they never develop toxic drugs! Why develop chemicals that are toxic for our planet?

Another version... work directly with industry

- Does industry want to make products that they have to withdraw years later?
- Do they want to face litigation?..asbestos an example
- Better to have a rational screen for environmental safety before letting them out of the bottle
- Or green chemistry...modify so doesn't accumulate but retains good properties

Yogi Berra's lesson....can we
ever learn



"This is like deja vu all over again."