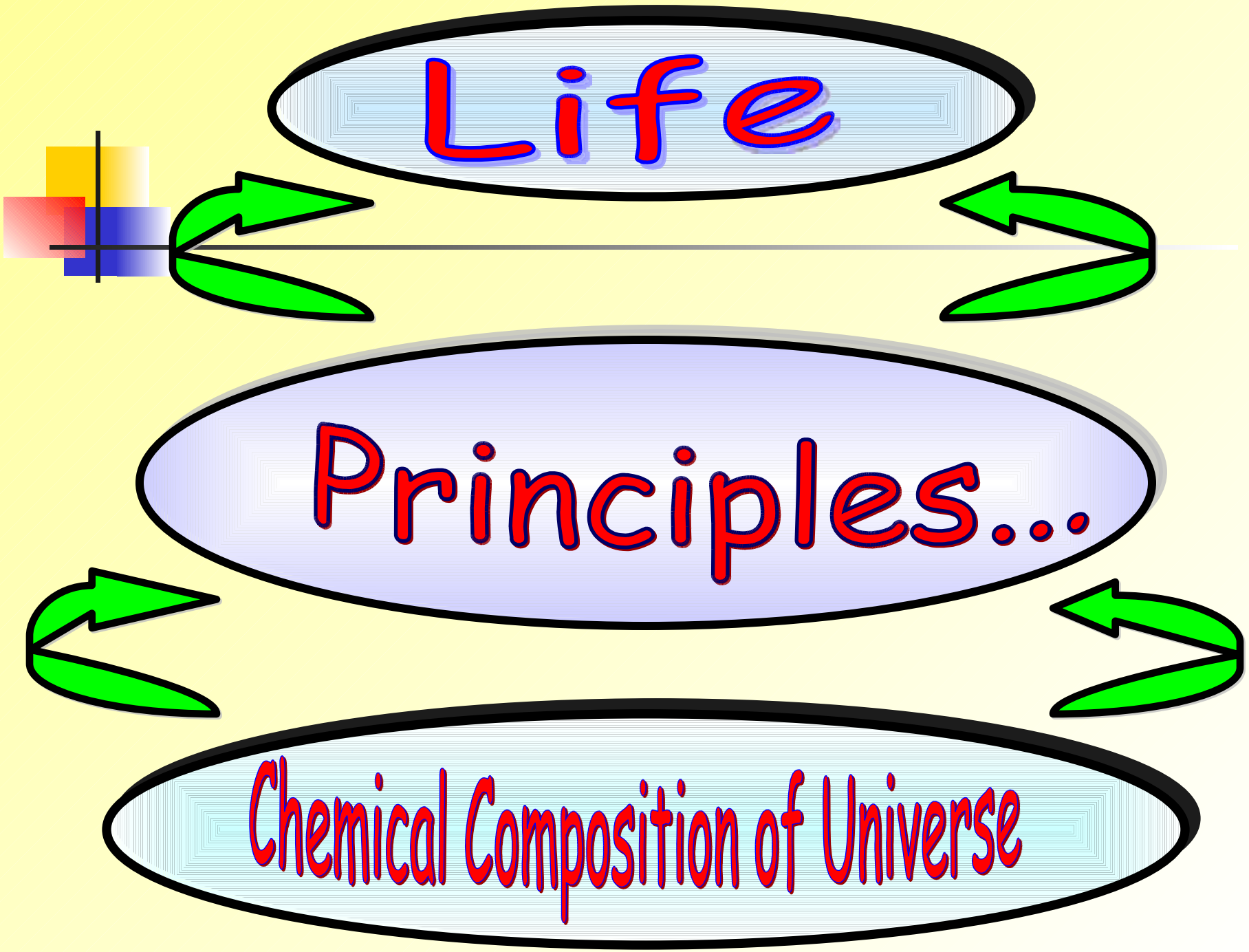
.....from Sequence to Survival

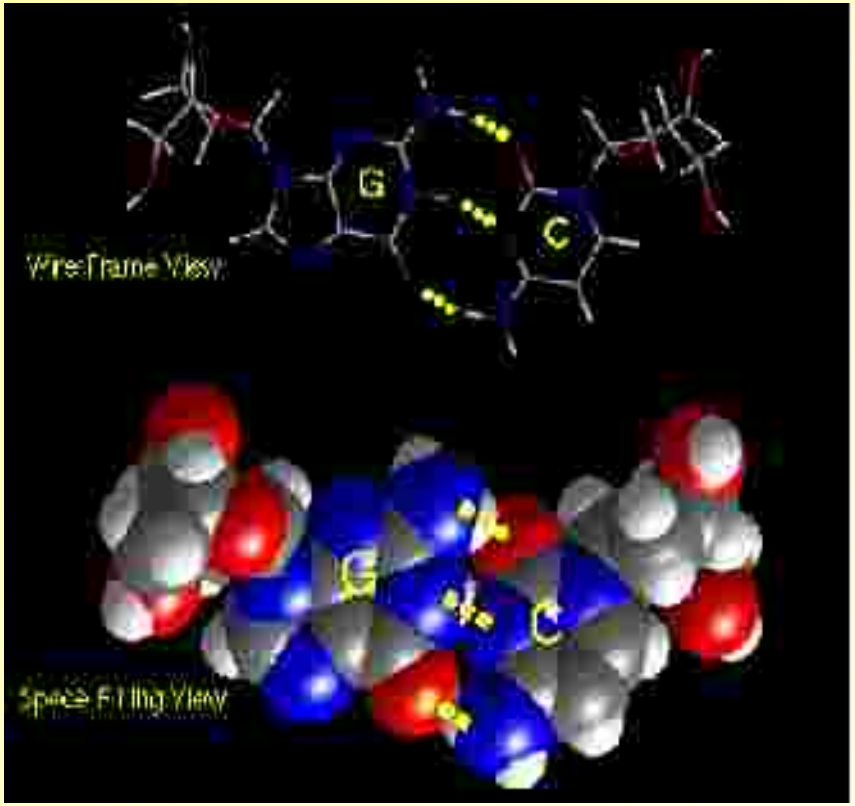
Vijay Bhatnagar

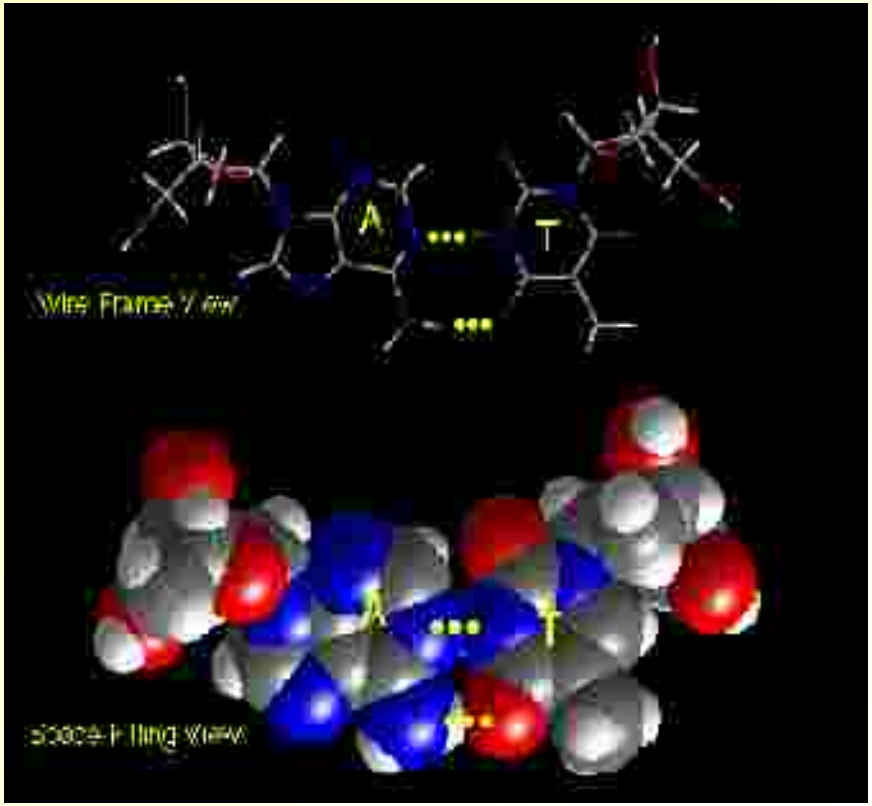
Assistant Director

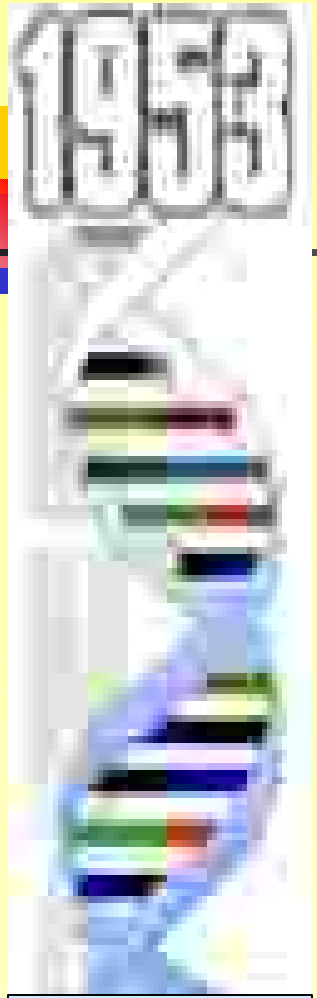
NIOH, Ahmedabad


Life
Principles...
Chemical Composition of Universe

H...O....C.....N.....P.....S....

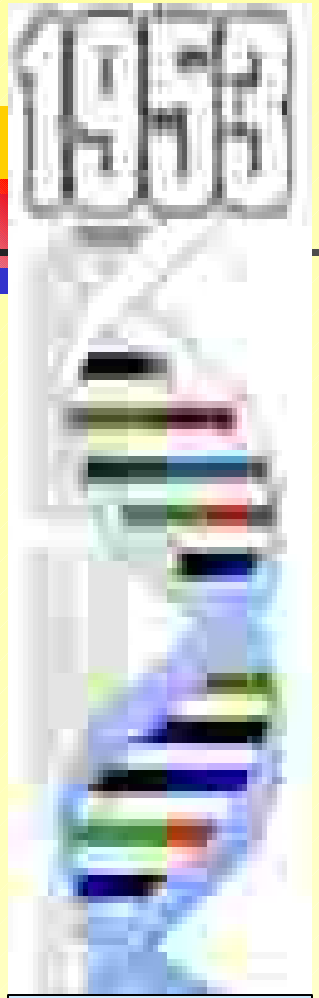

G
C
Wire Frame View
G
C
Space Filling View

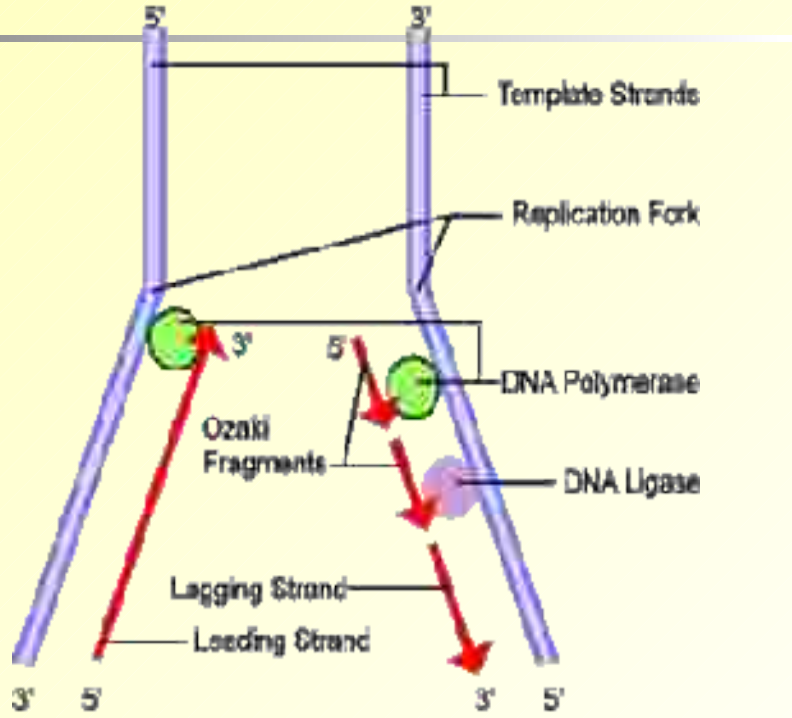

A
T
Wire Frame View
A
T
Space Filling View

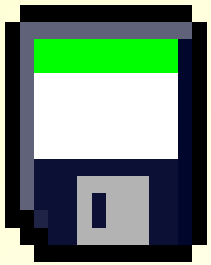


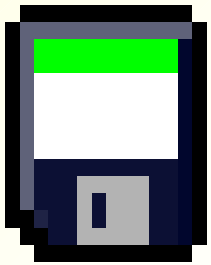
2006

Replication of DNA



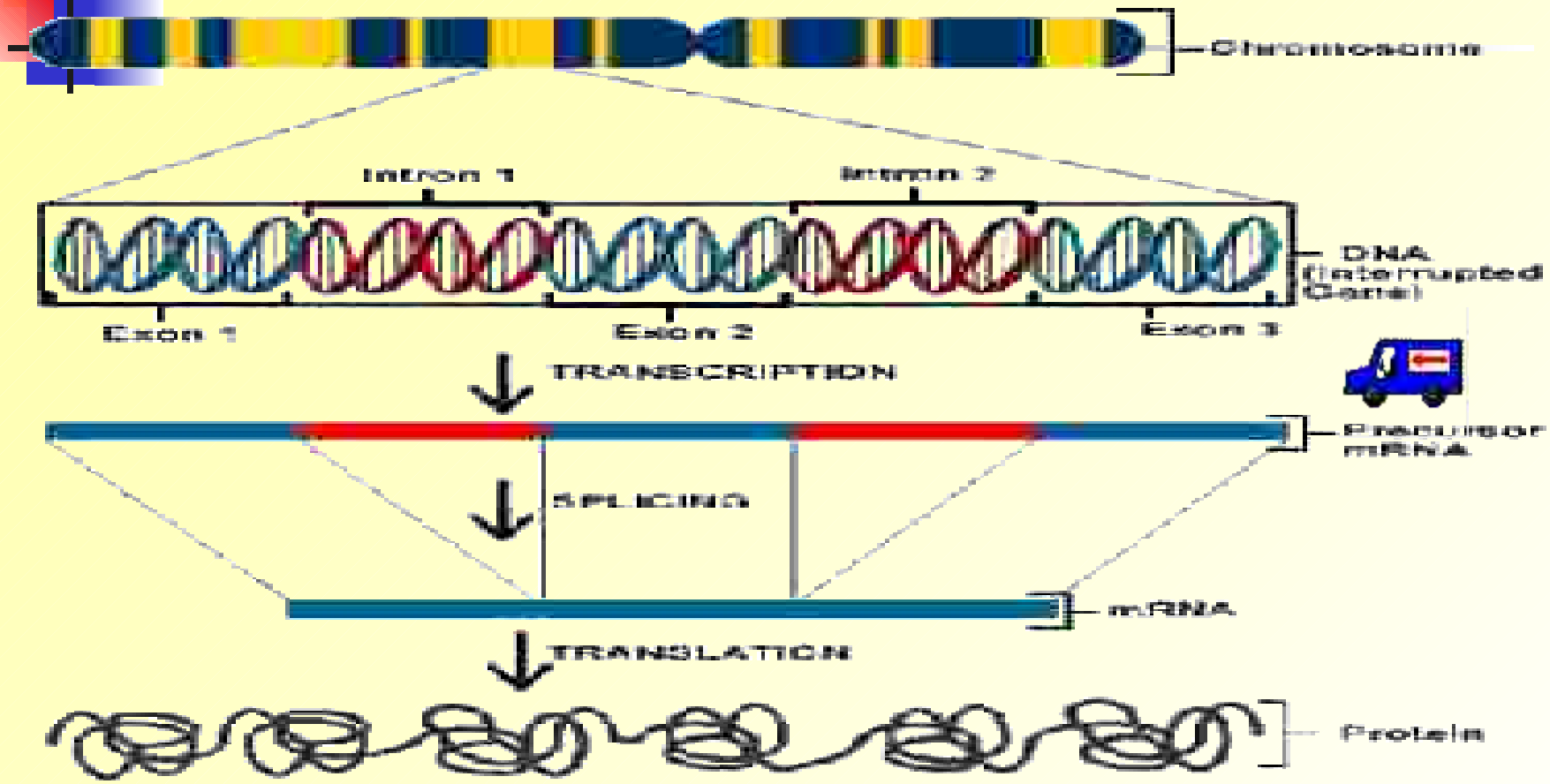


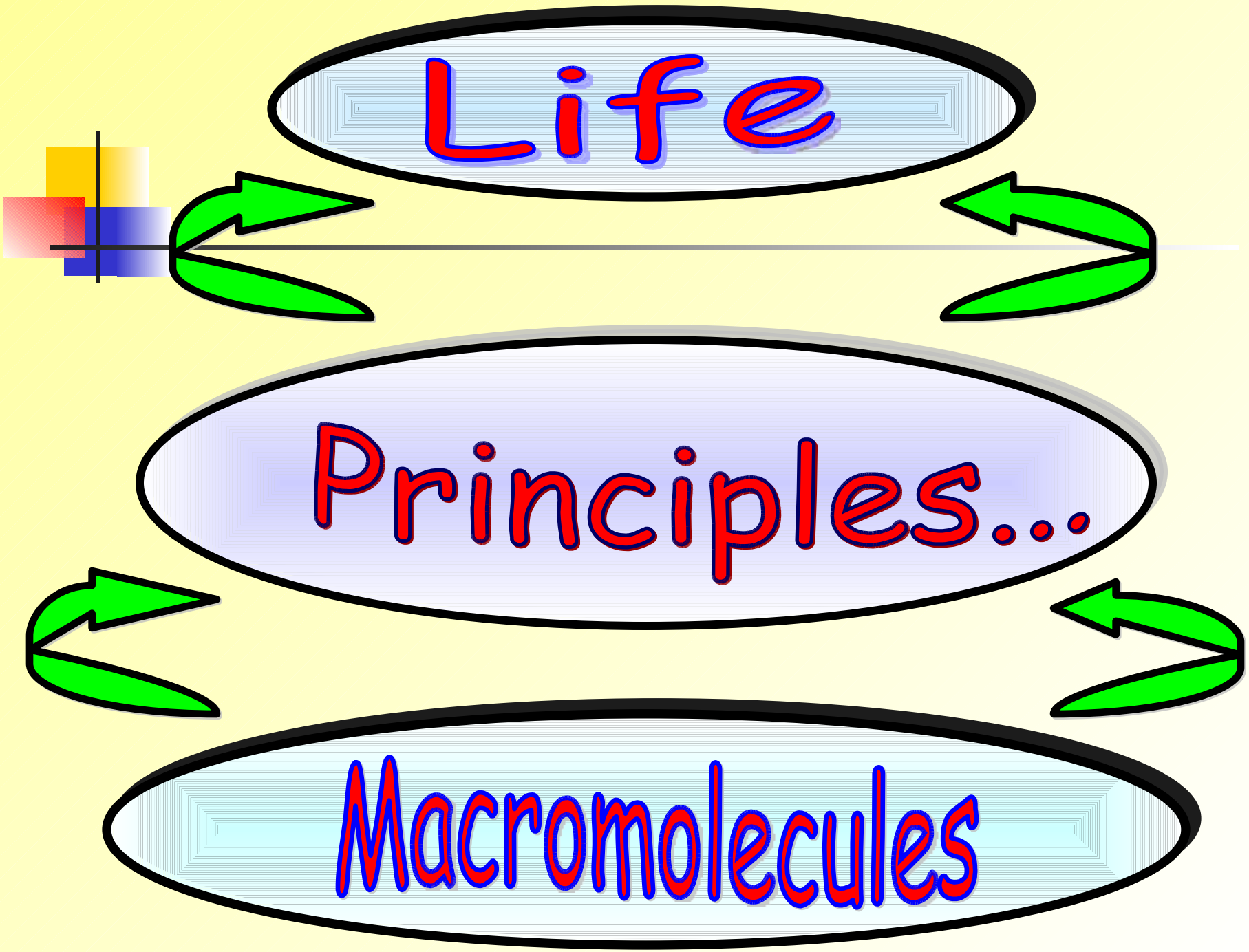


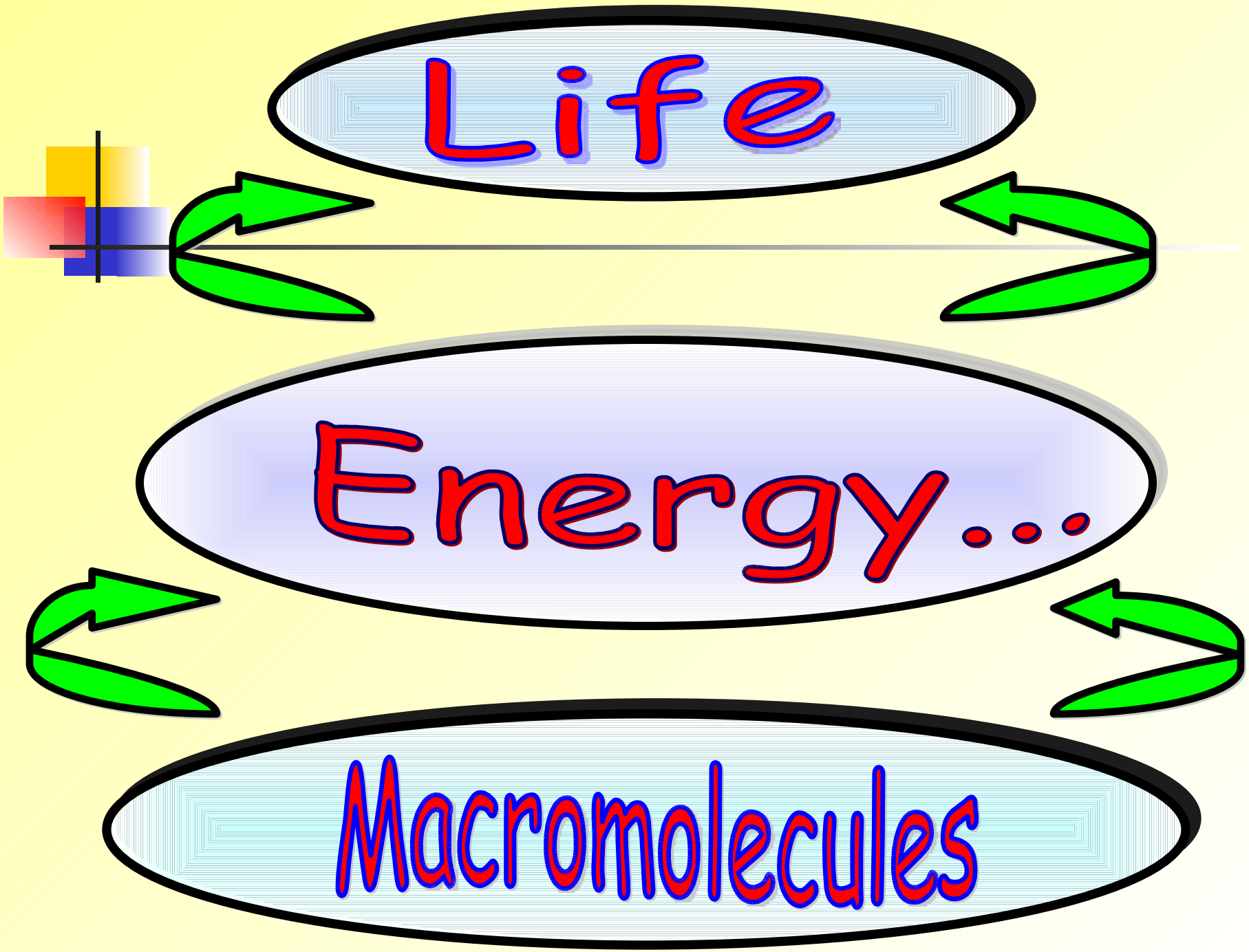


2006

DNA → RNA → PROTEIN




Life
Principles...
Macromolecules


Life
Energy...
Macromolecules

Life

Lord says the weapons can't reach the Soul,
Flame burns it not, waters cannot overwhelm,
Nor dry winds wither it. Soul is Impenetrable,
Unentered Unassailed, Unharmed, Untouched,
Immortal, Stable, Invisible, Ineffable by Words.

Macromolecules

Life

"Verily, all things have We created by measure" and "Everything to Him is measured.“ and He says, "And We have produced therein everything in balance.“ In the universe there is enormous diversity and variety of form and function. In it and its various elements there is fulfillment of man's welfare and evidence of the Creator's greatness;

Macromolecules

H...O....C.....N.....P.....S....

.....118 in PT

4.5 million / 100,000 / 9,000 / 1,000

Environment

- Concept
- Management
- EIA, …..alism

Environmental Problems

Global Warming, Acid Rain & O_3

Pesticides, PAHs, Phthalates, VOCs, Industrial Chemicals

Soil,Water Resources & Oceans

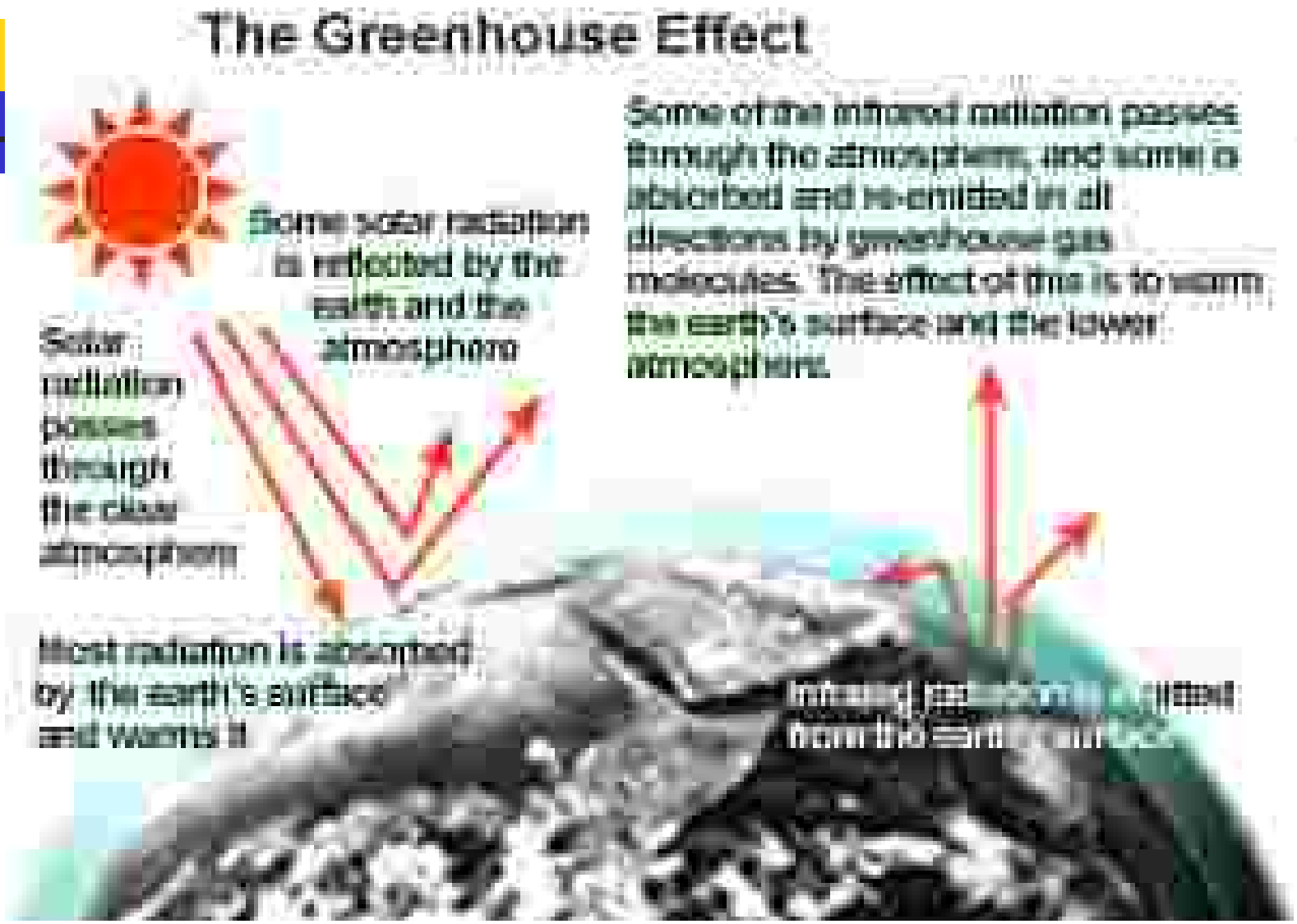
India

64 cities
6,141 towns
6,40,100 villages
30,00,00,000 houses
3.29 million sq meter land
Over 1.06 billion population
70 % population in villages

Policy (48A, 51A), Acts

40 % in single room dwellings
61 % lack drinking water

Global Warming



Earth's sunscreen – the ozone layer

In the stratosphere, thin layer of O_3 plays a vital role in absorbing UV radiation from the sun. It's level have been threatened by industrial activities and the related health effects are

Skin Cancer, Cataracts, and Immune Suppression

O_3 depletion Substances

Chlorofluorocarbons (CFCs)

(Refrigerants and insulating foams)

Methyl bromide

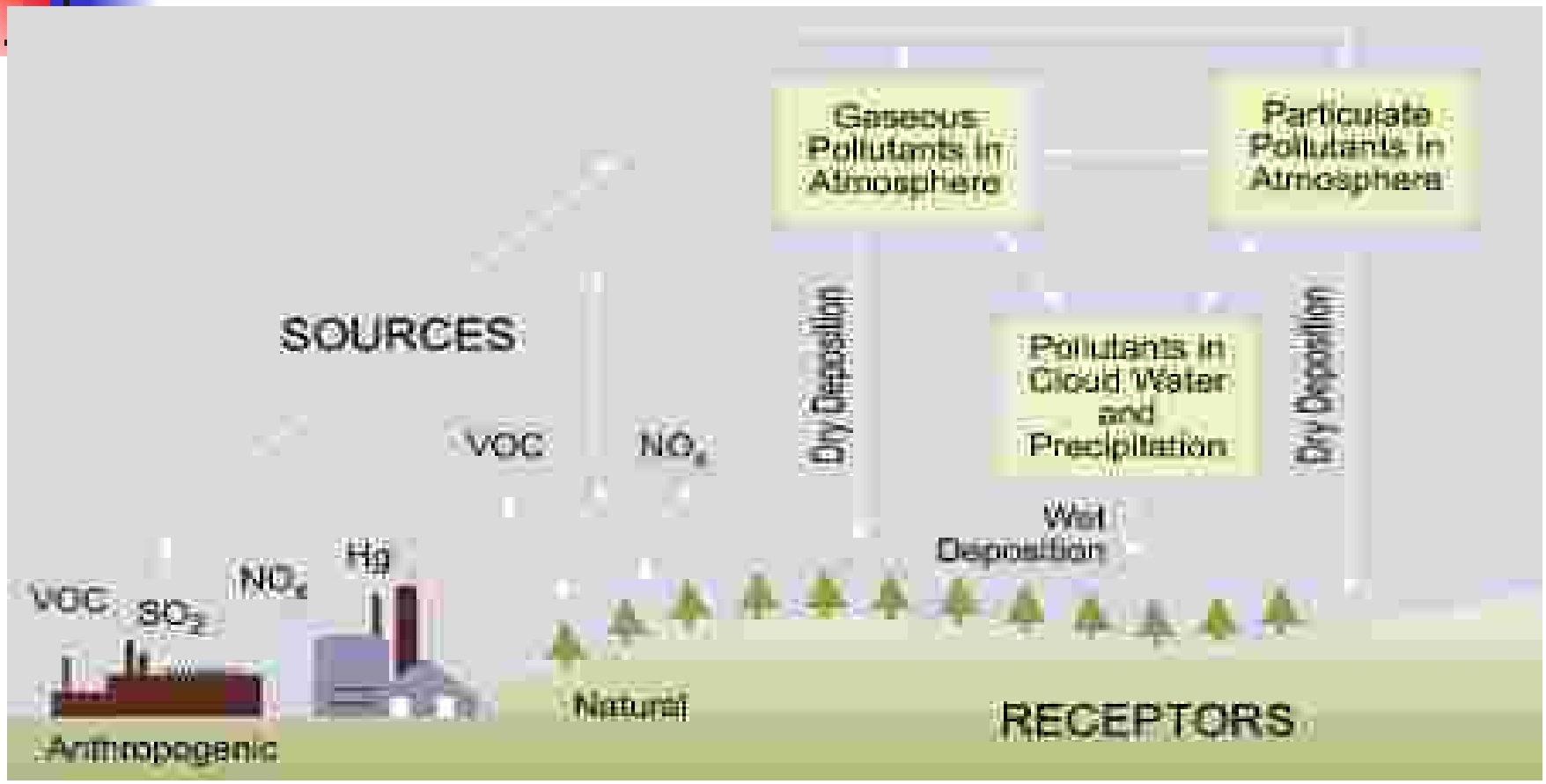
Halons

(Fire extinguishers)

Methyl chloroform

(Various industrial processes)

Acid Rain



Elevated levels of fine particles result premature death from heart and lung disorders, such as asthma and bronchitis.

Pest

Pesticides

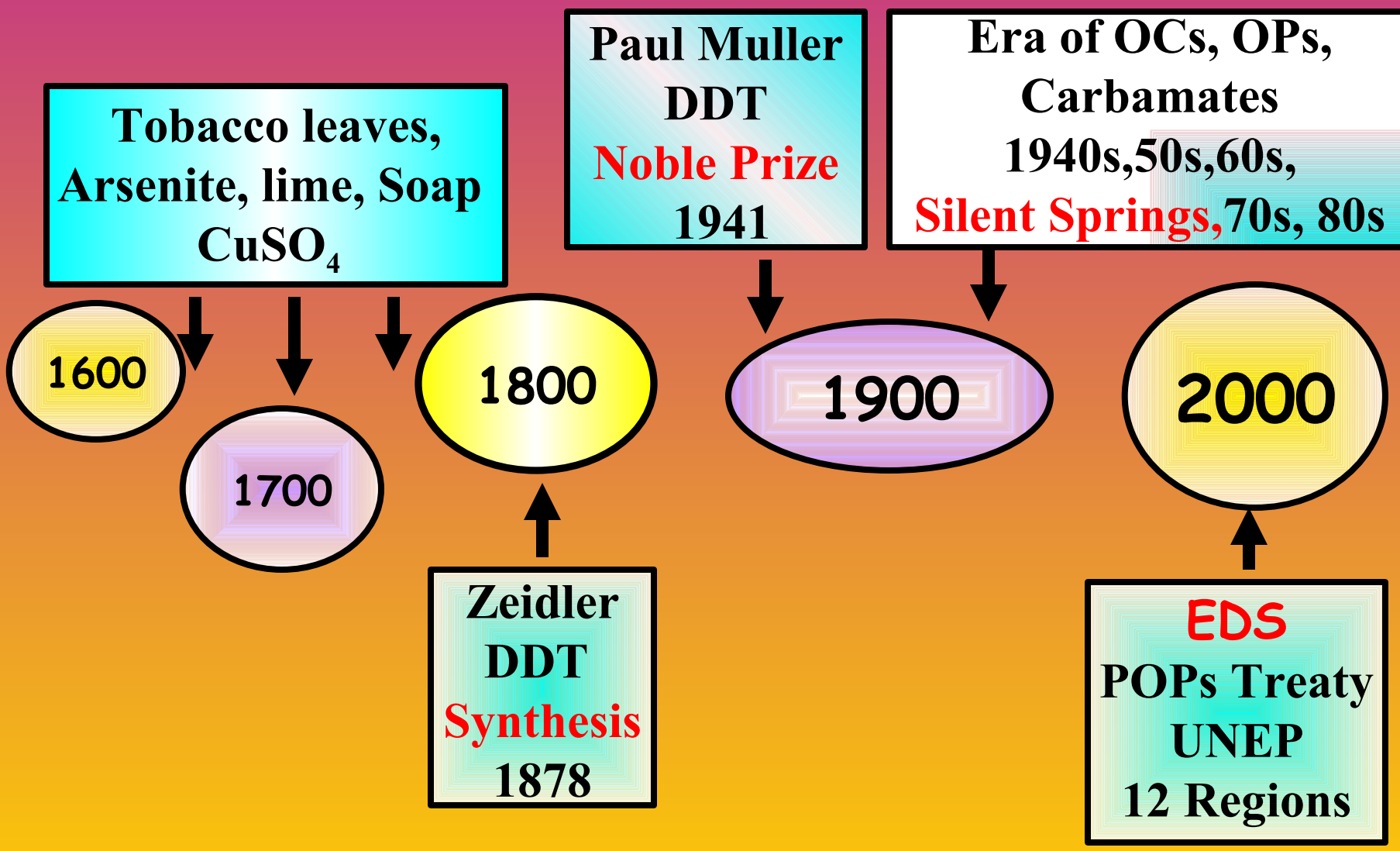
Insecticide Fungicide Algicide Herbicide

Rodenticide Bactericide Mollucide

Avicide Ovicide Defoliant Piscicide

Miticide Acaricide Attractant Nematodes

.....Journey


Tobacco leaves,
Arsenite, lime, Soap
$CuSO_4$
1600
1700
1800
Zeidler
DDT
Synthesis
1878
Paul Muller
DDT
Noble Prize
1941
1900
Era of OCs, OPs,
Carbamates
1940s,50s,60s,
Silent Springs,70s, 80s
2000
EDS
POPs Treaty
UNEP
12 Regions

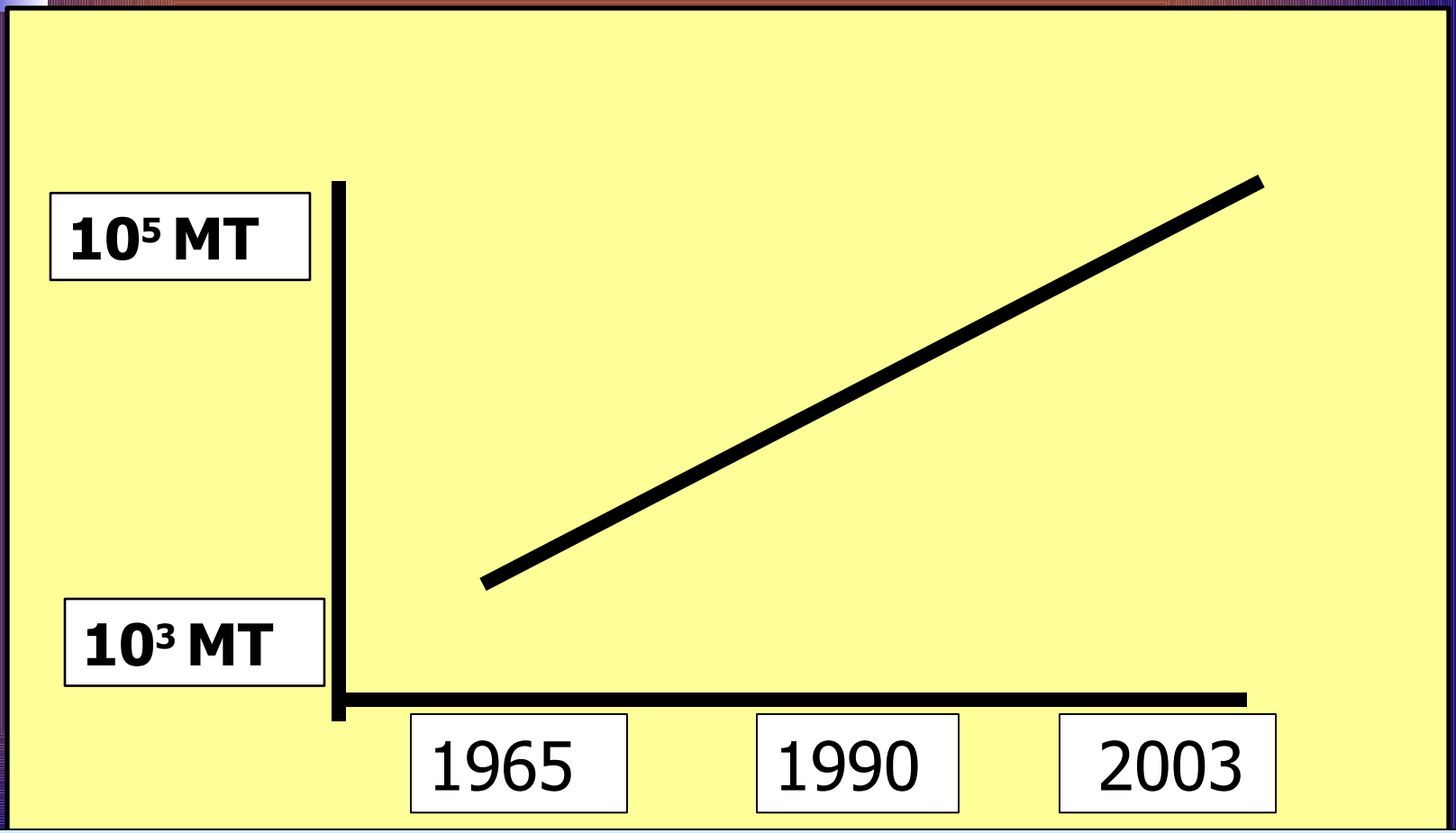
Structure of Pesticide Industry

Manufacturer

Formulators

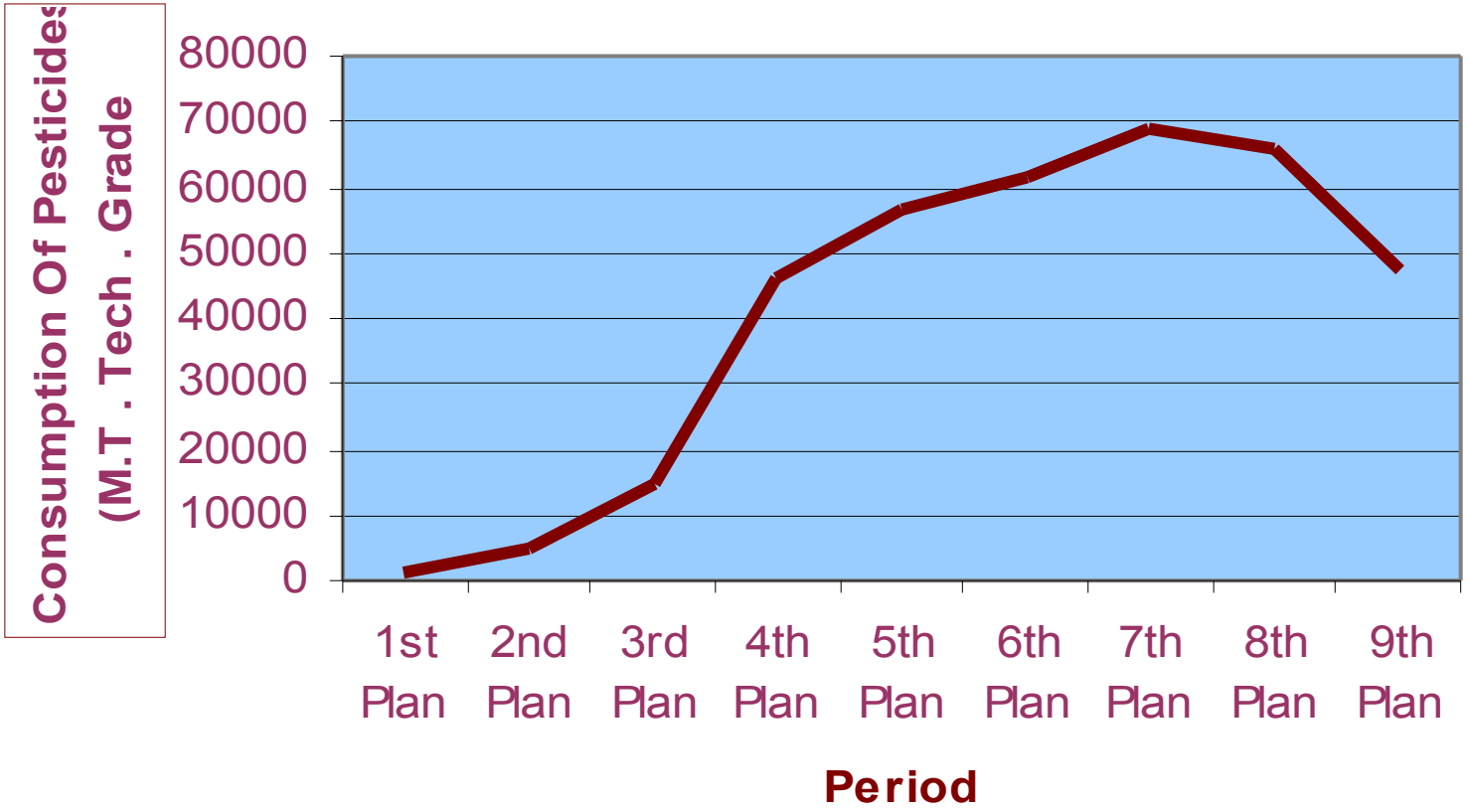
Dealers

Production of Pesticides



Thanks to Dr. Kanungo, MOHFW for his Presentation

Consumption Of Pesticides



Thanks to Dr. Kanungo, MOHFW for his Presentation

Persistent Organic Pollutants

- Aldrin, Chlordane, DDT, Dieldrin, Endrin, HCB, Heptachlor, Mirex and Toxaphene

- Polychlorinated Biphenyls (PCBs)
  Dioxins and Furans

Persistent Organic Pollutants

Resistant to Degradation
Propensity for Long Range Transport

Impaired Hepatic Metabolism
Reproductive abnormalities
Diminished Intelligence
Immuno-suppression
Yusho Rice Disease
Chloracne,
Suspected Human Carcinogens
Endocrine disruption etc

Persistent Organic Pollutants

UN at Helm

Global Treaty (RBA – IOR VI)

- **Anthropogenic Transport**
- **Monitoring Residues in Ecosystem**
- **Inventorization of Sources of Emission**
- **Formulation of Prevention Strategies**
- **Risk Assessment, Health Effects**

DOE Steering Meeting

- **Phasing out use of PCB containing equipments by 2025 & disposal by 2028**

Paucity of Data

Carcinogenecity of Pesticides

- **Captafol and Ethylene dibromide**

  - IARC Group 2A

- **Amitrole, Aramite, Atrazine,Chlordane, Chlorophenols, Chlordecone, Chlorphenoxy-herbicides, DDT, Dichlorovos, Heptachlor, HCB, HCH, Mirex, Nitrofen, PCP etc**

  - IARC Group 2B

Source: IARC Monographs, Volume 1-63 (1972-1995)

Carcinogencity of Pesticides

High serum DDE level have 4 fold risk of developing breast cancer (Wolff et al 1995)

Increase in lung cancer mortality observed in chlordane & other OC manufacturers and agricultural applicators (Wanget al 1979; Barthel et al 1981; Wong et al 1984; Blair et al 1983)

There is consistence evidence for an association of NHL with herbicides exposure (2,4-D, 2,4,5-T) and chlorophenols. (Rom 1992; Hoffmann 1996)

Congenital Malformation in Pesticide exposed Population

Finland	Hemminki (1980)	Musculoskeletal defects	OR = 5.0
New Zealand	Smith (1982)	Congenital defects	RR=1.2
Canada	McDonald (1988)	Congenital defects	OIE=2.6
Scotland	Sanjose (1991)	Low Birth weight	RR=1.4
Canada	Goulet (1991)	Still birth	OR=3.1
China	Zhang (1992)	Congenital defects	OR=1.8
Spain	Garcia(1998)	Congenital defects	RR=1.4

Ground Water contamination in WB (Zonal Office CPCB) during 2001 - 2002

	Durgapur (ppt)	Howrah (ppt)	Dhanbad (ppt)
Dieldrin	**ND - 39.3**	**ND - 12.3**	**ND - 64.3**
Lindane	**ND - 494**	**ND - 116.4**	**ND - 425.4**
Aldrin	**ND - 61.2**	**ND - 21.0**	**ND - 77.4**
DDT	**12.3 - 476**	**ND - 81.6**	**ND - 596.2**
Endosulfan	**ND - 134**	**ND - 12.3**	**ND - 243**
BHC	**ND - 190.4**	**ND - 201.3**	**ND - 15**
Hepatachlor	**ND - 44.1**	**ND - 13.8**	**ND - 62.4**

Pesticide Residues in Water Samples (1987) from Ahmedabad, India*

Water body	No of samples	Pesticide	Range (ng/L)
Municipal Supply	60	Total HCH	23.9 – 2488.7
		Total DDT	10.9 – 314.9

*Jani et al. *Bull Environ Contam Toxicol 47:* 381, 1991.

Pesticide Residues in Water Samples (1993) from West Khasi Hills. Meghalaya, India*

Water body	No of samples	Pesticide	Range (mg/L)
Ground Water	39	HCH DDT Aldrin	0.012 – 0.0118 0.0077 – 0.433 ND – 0.04
Surface water	32	HCH DDT Aldrin	0.199 – 0.059 0.0096 – 0.38 0.0019 – 0.044

*Kumar & Singh. *Indian J Environ Prot 13*: 349, 1993.

Pesticide Residues in water system in and around Bhopal (1990), India*

Sample Source	Total HCH (ppm)	Total DDT (ppm)
Wells	4.654	5.794
Hand pumps	6.13	14.548
Ponds	9.941	16.059

*Dikshith et al. *Bull Environ Contam Toxicol 45:* 389, 1990.

Pesticide Residues in Rain Water Samples (1992) in Haridwar, India*

Month	Total HCH (ug/L)
January	0.113
February	0.060
June	0.152
July	0.095
August	0.032
September	0.008
Average	0.077

*Dua et al. *Bull Environ Contam Toxicol* 52: 797, 1994.

Pesticide Residues in Tap water near vicinity of lakes in Nainital (1994), India*

Lakes	Total HCH (ug/L)	Total DDT (ug/L)
Kuurpatal	3.782	17.014
Sattal	2.884	8.767
Bhimtal	2.629	5.982
Naukuchaital	1.183	2.749
Nainital	1.756	15.822

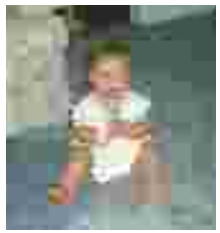
*Dua et al. *Bull Environ Contam Toxicol 60:* 209, 1998.

Pesticide Residues in Water Samples (2000) in Jaipur, India*

Water body	No of samples	Pesticide	Range (ppb)
Tube well	30	HCH	0.52 – 12.23
		Dicofol	0.01 – 2.05
		Dieldrin	0.30 – 1.25
		alpha-Endosulfan	0.47 – 3.01
		Chlorpyriphos	0.96 – 1.76
Open well	38	HCH	0.3 – 37.17
		Dieldrin	0.2 – 3.32
		Endosulfan Sulfate	5.97 – 13.54
		Chlorpyriphos	0.42 – 1.36
Lake	24	g-HCH	0.55 – 6.04
		alpha-Endosulfan	0.01 – 15.0
		Chlorpyriphos	0.44 – 1.10

*Singh B, Gupta A. *Bull Environ Contam Toxicol 69:* 49, 2002.

Levels of DDT and HCH Residues in Human Milk in General Population in India



City	Year	#	Whole Human milk basis	
			DDT (ppm)	HCH (ppm)
Ludhiana	1979	75	0.51	0.195
Lucknow	1980	25	0.12	0.107
Ahmedabad	1981-82	50	0.305	0.224
Bangalore	1984-85	6	0.05	0.01
Calcutta	1984-85	6	0.11	0.03
Karnal	1984-85	6	0.19	0.03
Bombay	1984-85	6	0.22	0.05
Delhi	1985-86	60	0.344	0.38
Delhi	1994	25	1.27	0.327
Haridwar	1996	-	0.021	0.027
Bhopal	2002	12	-	0.104

Bhatnagar VK, (2003) In Proc "*Symposium on Risk Assessment of Pesticide Residues in Water & Food*"; Sponsored ICMR & ITRC, Publ ILSI-India

Levels of DDT and HCH Residues in Human Fat in General Population in India

City	Year	#	Human Blood DDT (ppm)	Human Blood HCH (ppm)
Delhi	1964	35-67	26.0	1.43
Delhi	1973	94	21.8	-
Delhi	1976	14	4.7	-
Chandigarh	1980	10	20.03	2.44
Agra	1980	14	12.02	2.0
Bombay	1980	34	6.15	1.61
Calcutta	1980	45	6.5	1.61
Bhopal	1980	14	9.14	1.06
Ahmedabad	1980	80	21.81	3.87
Bangalore	1980	116	7.82	5.05
Meerut	1980-81	32	8.14	-
Delhi	1982	340	22.25	16.85
Ahmedabad	1992	12	3.96	4.05

Bhatnagar VK, (2003) In Proc "*Symposium on Risk Assessment of Pesticide Residues in Water & Food*"; Sponsored ICMR & ITRC, Publ ILSI-India

Levels of DDT and HCH Residues in Human Blood in General Population in India

City	Year	#	Human Blood DDT (ppm)	Human Blood HCH (ppm)
Delhi	1975	103	0.17 - 0.68	-
Lucknow	1980	25	0.02	0.022
Lucknow	1982	48	0.028	0.075
Delhi	1982	340	0.71	0.49
Delhi	1985	50	0.301	-
Haridwar	1992	37	0.02	0.021
Ahmedabad (Rural)	1992	31	0.048	0.148
Ahmedabad (Urban)	1992	10	0.21	0.07
Delhi	1994	25	0.271	0.05
Allahabad (Rural)	1996	50	-	0.152
Ahmedabad (Urban)	2003	18	0.032	0.041

Source: Bhatnagar VK, (2003) In Proc "*Symposium on Risk Assessment of Pesticide Residues in Water & Food*"; Sponsored ICMR & ITRC, Publ ILSI-India

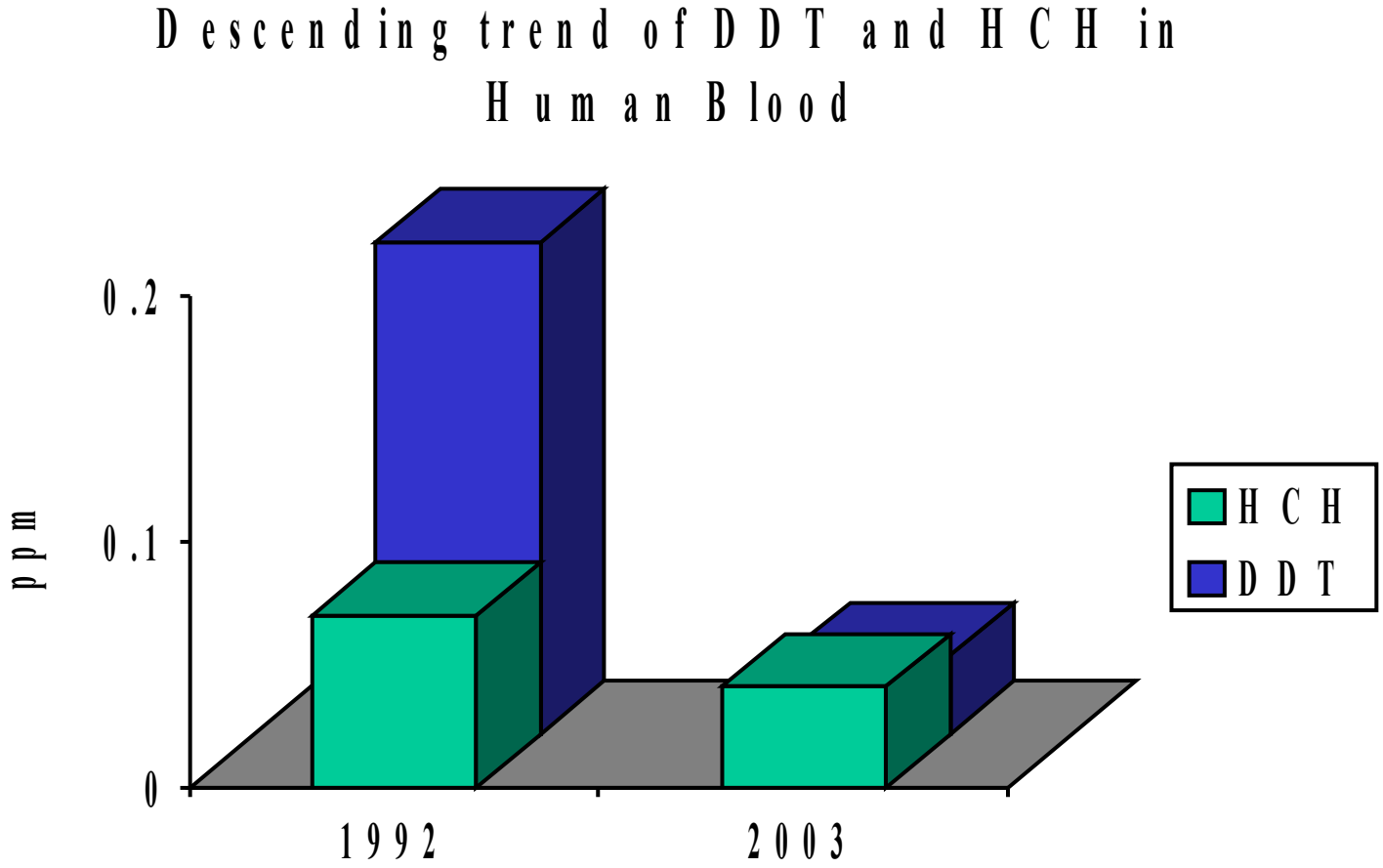
Pesticide Residues in Human Blood samples from Ahmedabad

Compound	No	Mean (ppb)	Range (ppb)
pp´-DDE	18	20.85	10.43 – 38.33
op´-DDT	15	1.15	0.42 - 0.12
pp´-DDD	18	2.03	0.77 – 4.43
pp´-DDT	17	9.28	3.66 – 24.06
Total DDT	18	32.61	21.17 – 54.47
α-HCH	18	4.49	1.00 – 9.16
β-HCH	18	35.06	20.11 – 82.09
γ-HCH	18	1.69	0.72 – 3.09
Total HCH	18	41.23	22.55 – 91.06
HCB	7	0.20	0.13 – 0.27

Bhatnagar V, Kashyap R, Zaidi S, Kulkarni P, Saiyed HN (2004)
Bull Environ Contam Toxicol 72: 261- 265

City	Year	#	DDT (ppm)	HCH (ppm)
Ahmedabad (Rural)	**1992**	**31**	**0.048**	**0.148**
Ahmedabad (Urban)	**1992**	**10**	**0.21**	**0.07**
Ahmedabad (Urban)	**2003**	**18**	**0.032**	**0.041**

Bhatnagar VK, Kashyap R, Saiyed HN (2006)
'Levels of OC pesticides in Ahmedabad' Asian Jour Chemistry 18(2)


Descending trend of DDT and HCH in Human Blood
ppm
0.2
0.1
0
1992
2003
HCH
DDT

High Risk Groups

- Malathion sprayers
- Pest control agency workers
- Phosphamidon workers
- Phorate formulators
- Cyfluthrin sprayers
- Malathion sprayers
- Phosalone workers
- BHC workers
- Methomyl sprayers
- Aerial spray of endosulfan
- Formulators

Studies on Methomyl Exposure

22 male subjects, sprayed 4-5 hr for 5 days

Pesticide related morbidity (28%), neurological (13%), GIT (14%)

Lowering in Plasma ChE

Cardiac toxicity: ECG changes (64%) T wave inversion and ST segment changes

Saiyed HN, Sadhu HG, Bhatnagar VK, Dewan A, Venkaiah K, Kashyap SK (1992) *Human Exp Toxicol* 11; 93 - 97

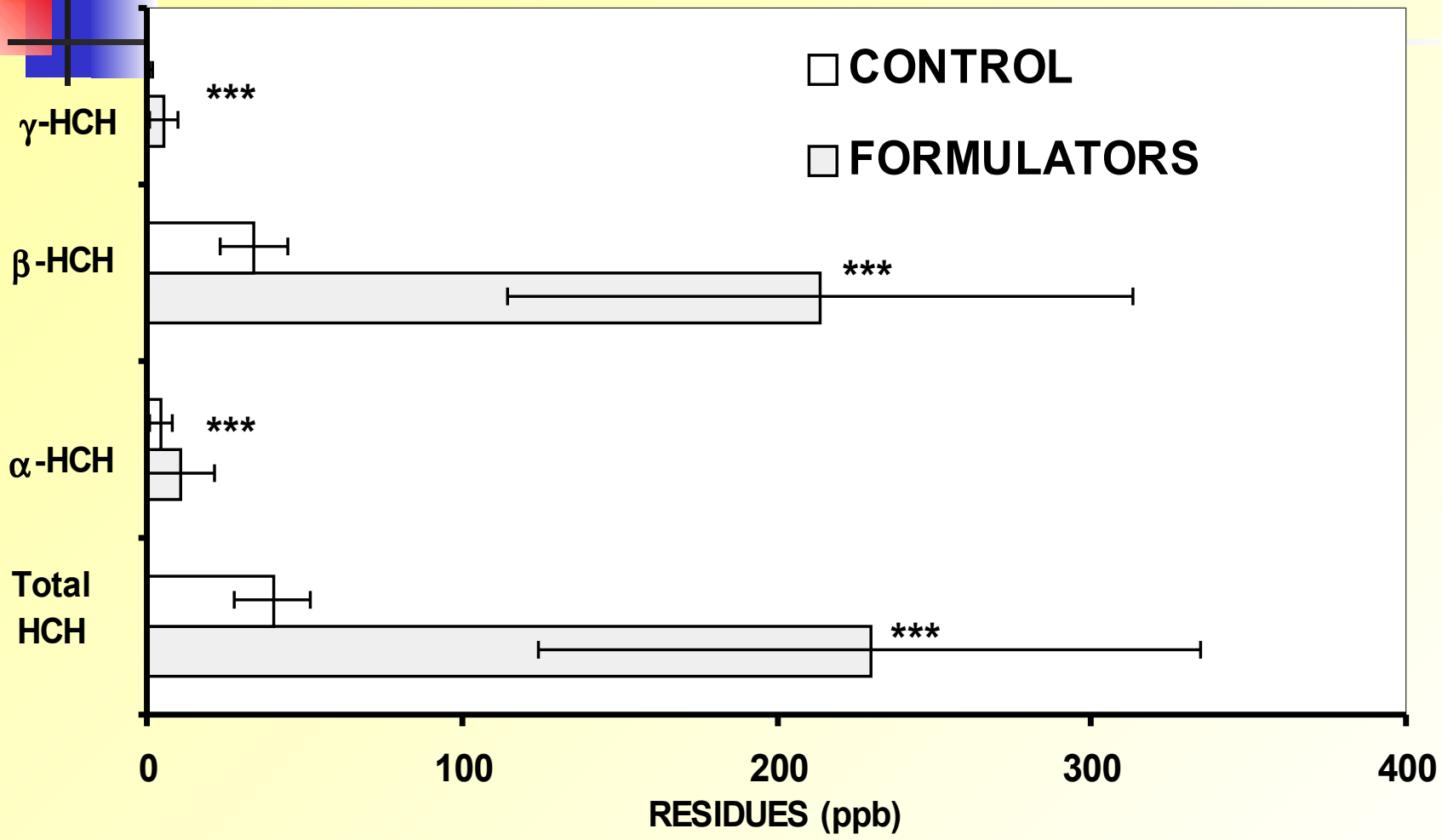
Study on Pesticide Formulators

- Formulators (n=30) of a unit of organized sector engaged in dust and liquid formulation of various pesticides (Lindane, Monocrotophos, Quinalphos & Phorate) and a control group (n=14) were enrolled.
- Informed consent and Questionnaire
- Biological samples were collected for analysis of residues and clinical parameters

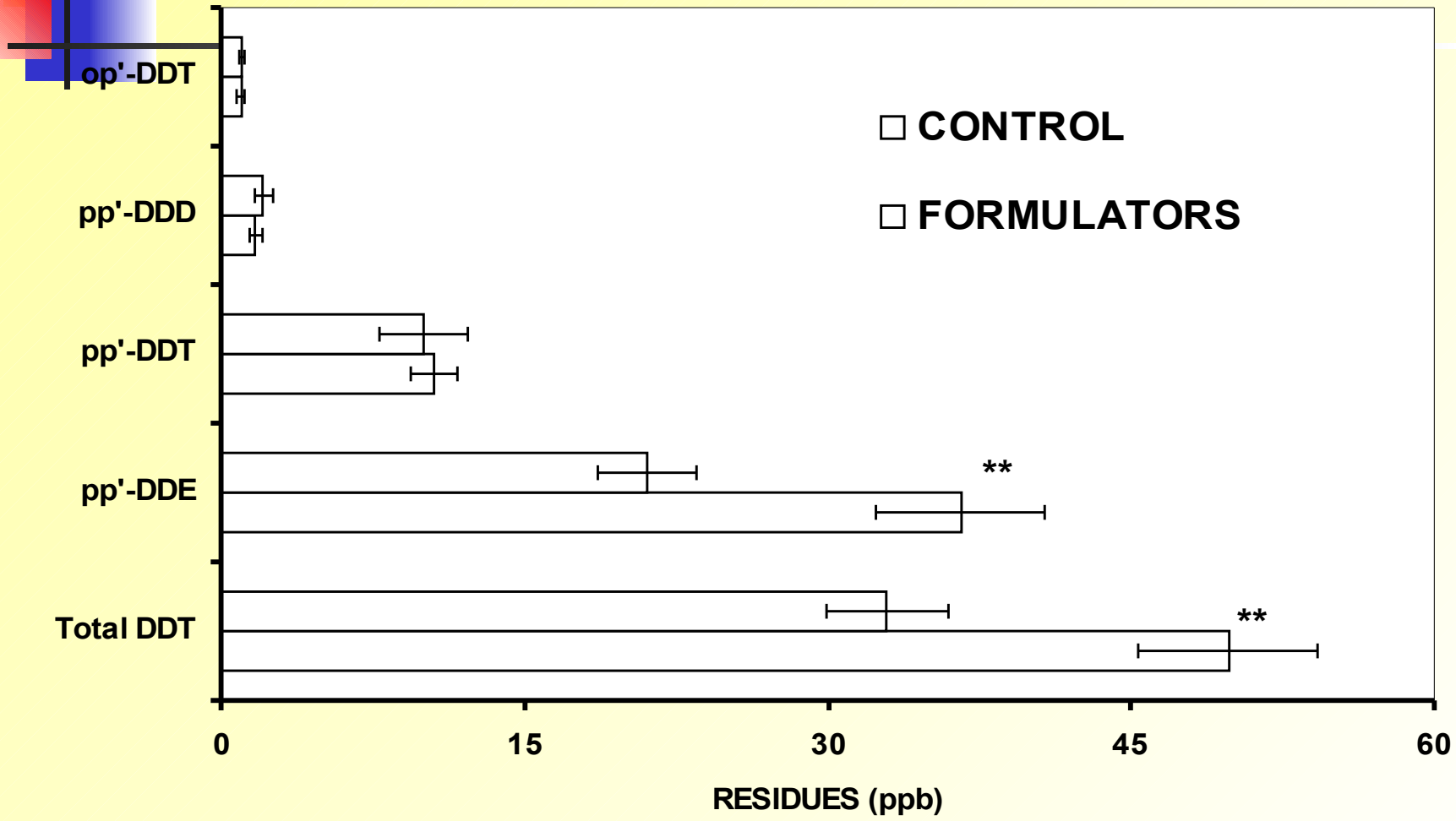
	Control N=14	Formulators (N=30)
HCH (ppb)	39.9 ± 12.1	229.7 ± 105***
ChE (IU/ml)	3.4 ± 0.2	2.2 ± 0.83***
IgM (mg %)	98.7 ± 18.4	116.6 ± 20.9*
IgG (mg %)	1163 ± 282.9	1297 ± 425.2
IgA (mg %)	206.5 ± 76.8	234.3 ± 80.84

Bhatnagar V, Karnik A, Suthar A, Zaidi S, Kashyap R, Shah M, Kulkarni P, Saiyed HN (2002)
Bull Environ Contam Toxicol 68: 22-28

HCH Residues in Control & Formulators



DDT Residues in Control & Formulators



Conclusion

- Data Gap
- Effective Monitoring & Surveillance
- Development of Safer Molecules
- Periodic Environmental Monitoring
- Cooperation

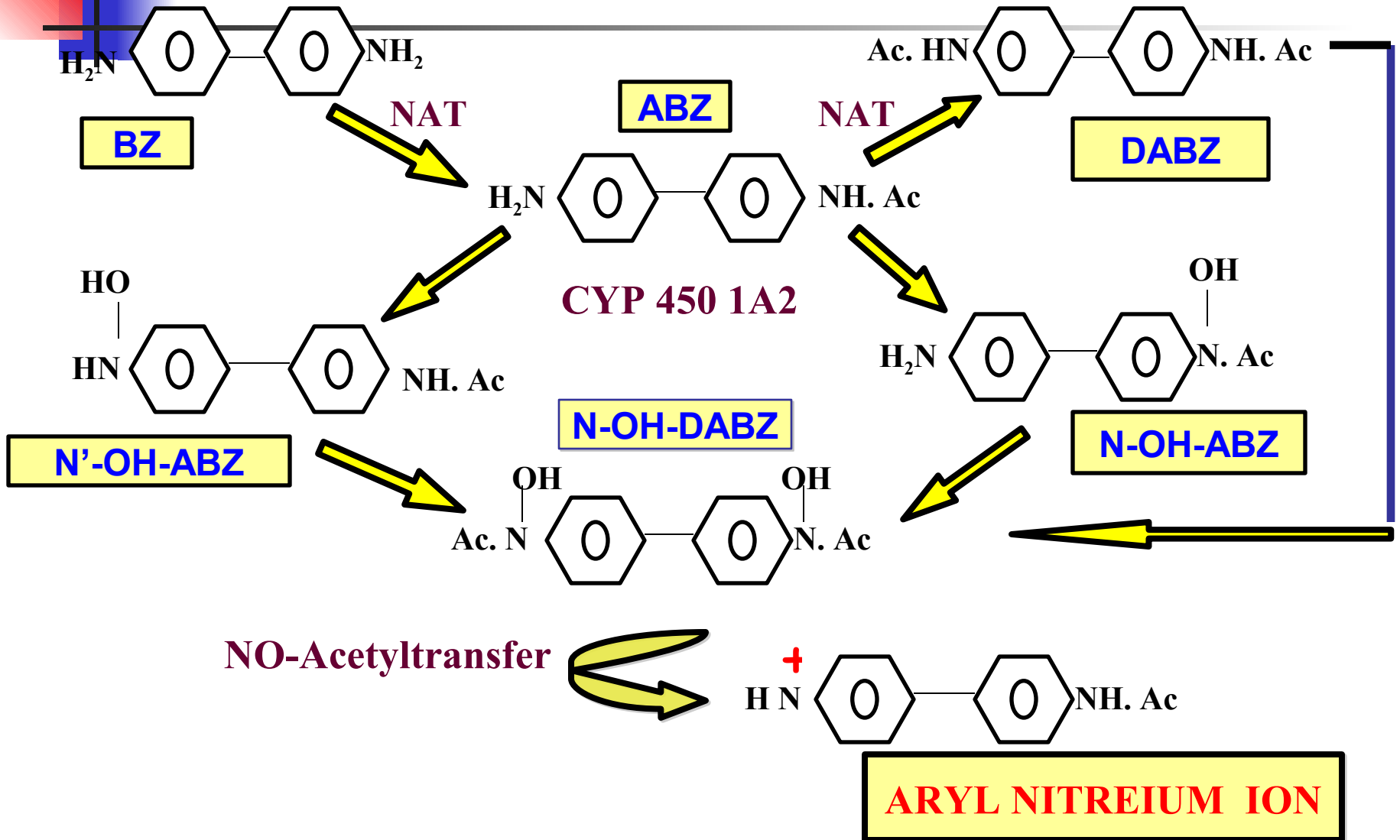
Industrial Chemicals

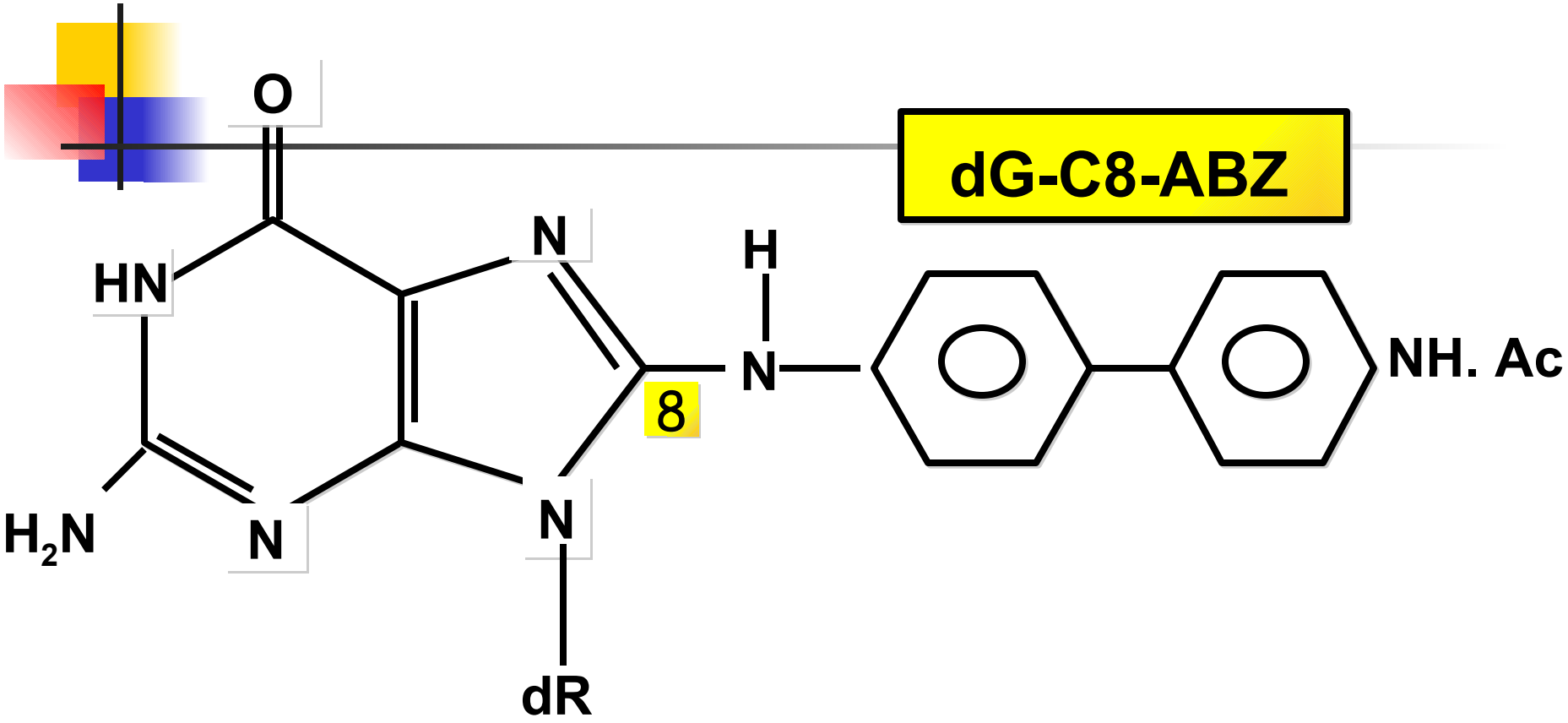
PAH: benzo(a)pyrene, benzo(e)pyrene, Chrysene,…….
Hetrocyclic PAH: Quinoline, Benzo(f)quinoline,…….
Nitro PAH: 1-Nitropyrene, 2-Nitropyrene,……..,
Cyclopenta-fused PAHs: Aceanthrylene, Acenaphthylene,……
Aromatic Amines: Benzidine, 2-Naphthylamine, Toluidine,
4-Aminobiphenyl, MOCA (plastic, rubber)
Alkylating Agents: Propylene oxide, Dicarbazine, Ethylene oxide,……
Dyes: Congo red, Evans Blue, Sudan 1,…….
Myotoxins: Aflatoxin B,…..
Chemicals Producing Cyclic Adducts: Vinyl chloride,Vinyl carbamate,
Pesticides: Alachlor, dichloflaunid

Studies on Benzidine (BZ) and BZ based Dyes

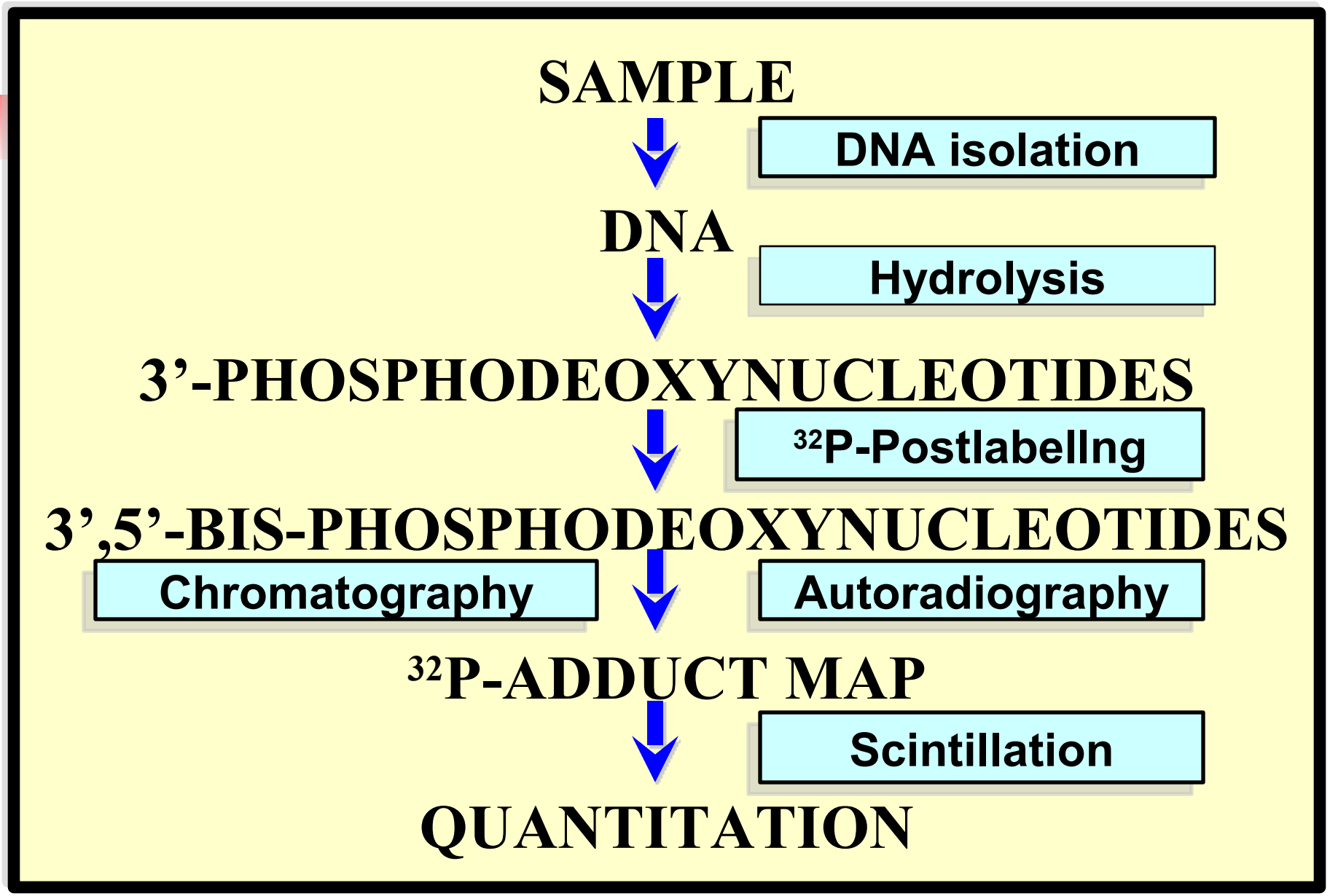
- A cross sectional study in BZ and BZ dye exposed workers (NIOH-NIH Collaboration)
- Study population: Exposed subjects – 33
  (BZ – 15; BZ dye exposed – 18)
  Control subjects – 15
  Questionnaire
  Biologic samples (blood & urine)
- BZ-DNA adducts by ^{32}P-post labeling
- BZ metabolites and Hb-BZ adducts by GCMS

Mechanism of Support




dG-C8-ABZ
O
HN
N
H
N
NH. Ac
8
H_2N
N
N
dR

DNA adduct analysis



BZ Metabolites analysis

Urine Sample

↓

Purification – C18 Column

↓

Extraction – Ethyl Acetate

↓

Reduction – $LiAlH_4$ / THF

↓

Derivatization – PFP / Toluene

↓

GCMS

Hb-BZ adduct analysis

Hb Sample

↓

Purification –Chem Elute Column

↓

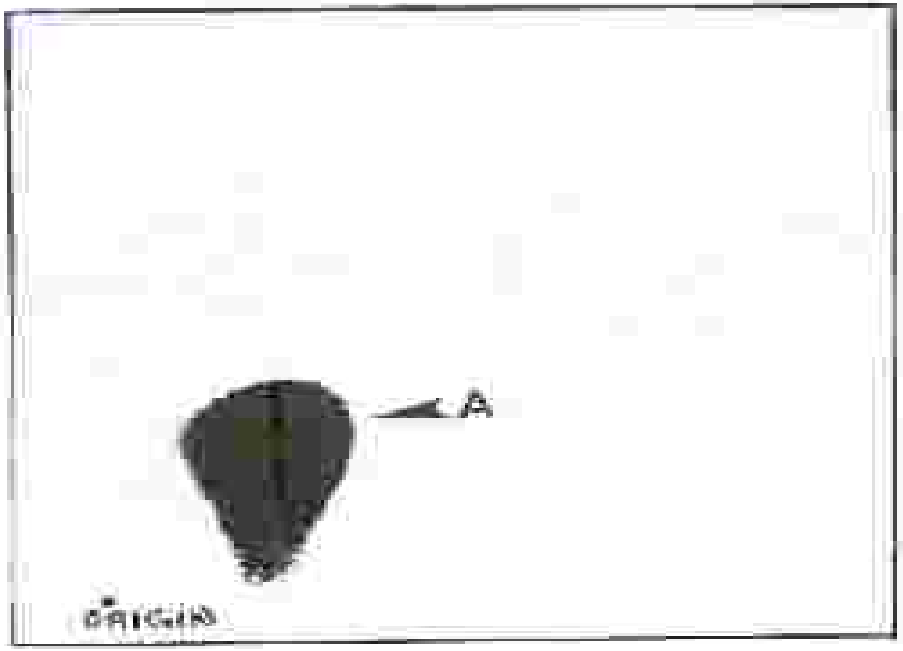
Alkaline Hydrolysis

↓

Derivatization – HFFA

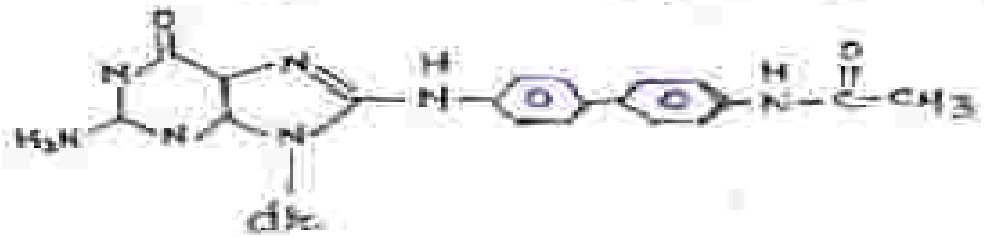
↓

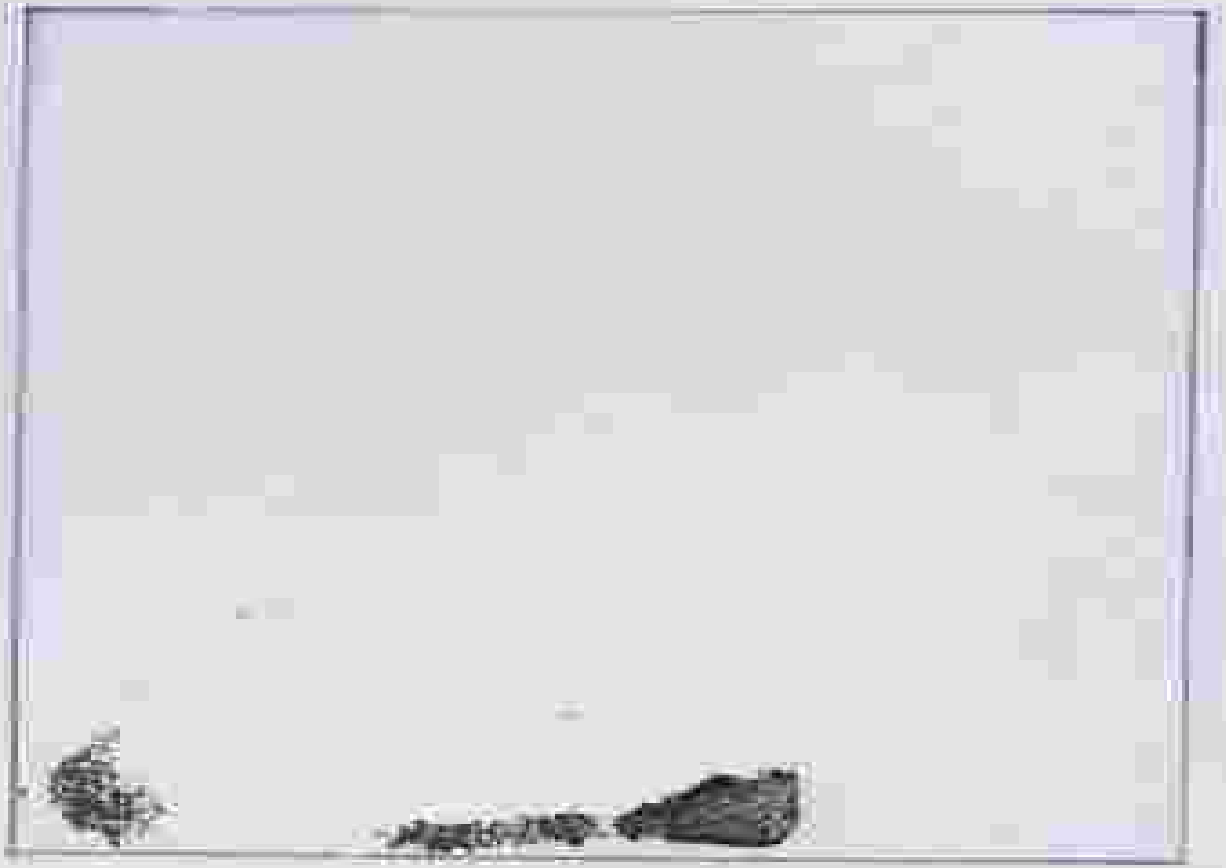
GCMS



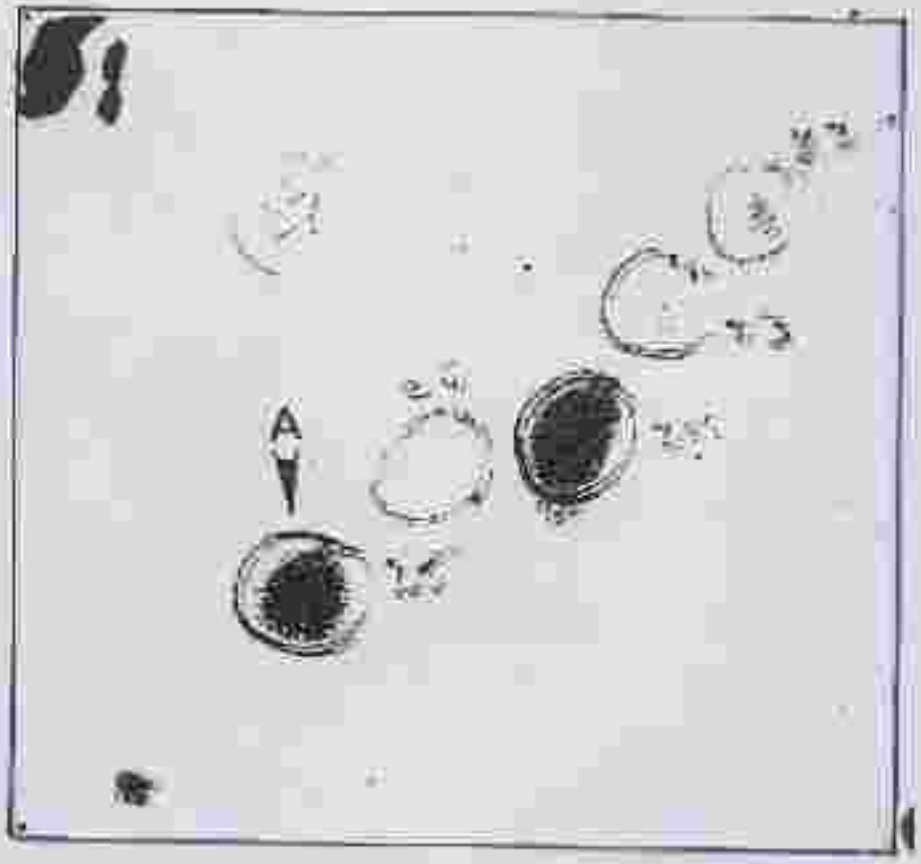
AUTORADIOGRAM of DNA-BZ ADDUCT

N-(DEOXYGUANOSIN-8-YL)-N'-ACETYLBENZIDINE

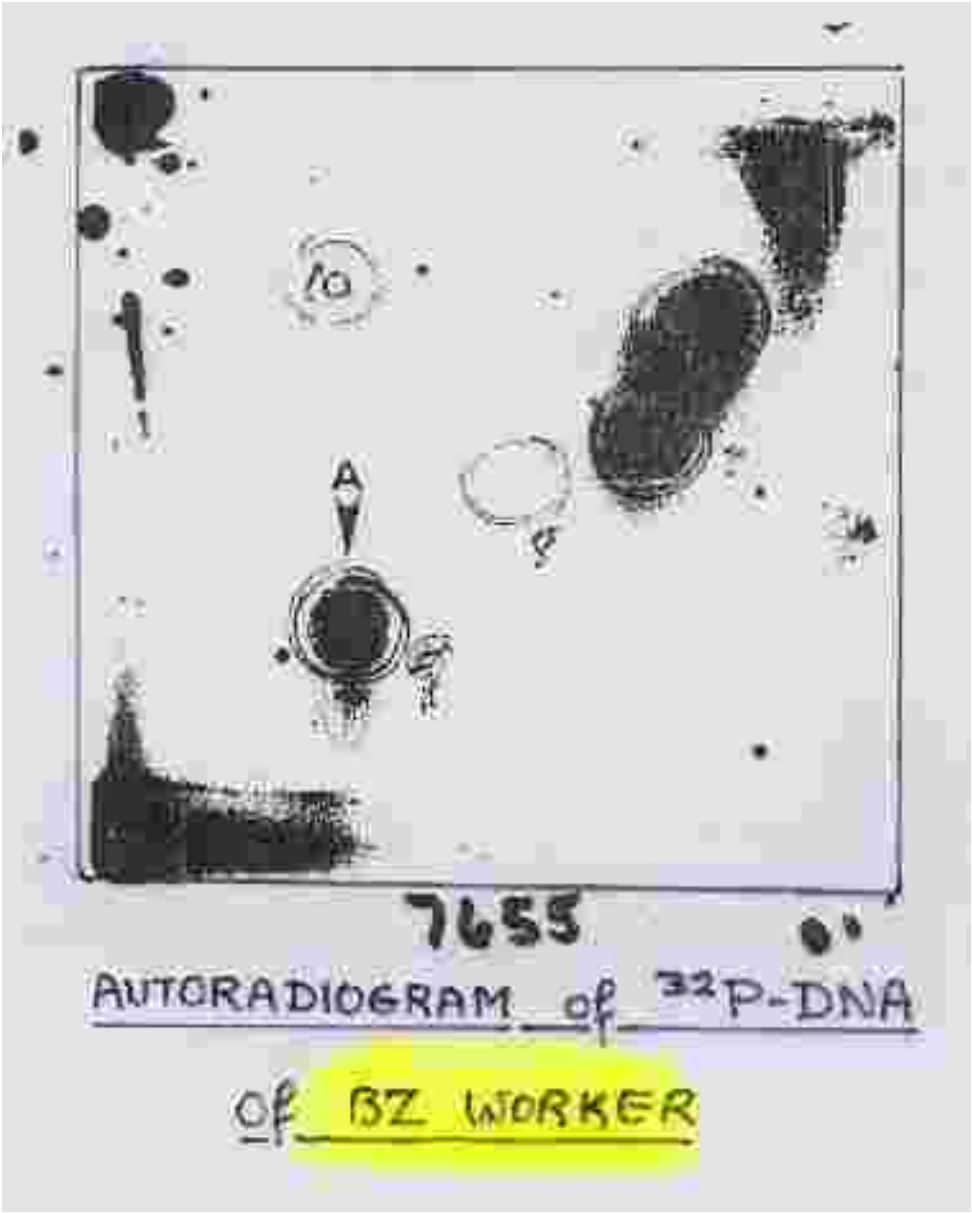




Autoradiogram of ^{32}P-DNA of a control subject



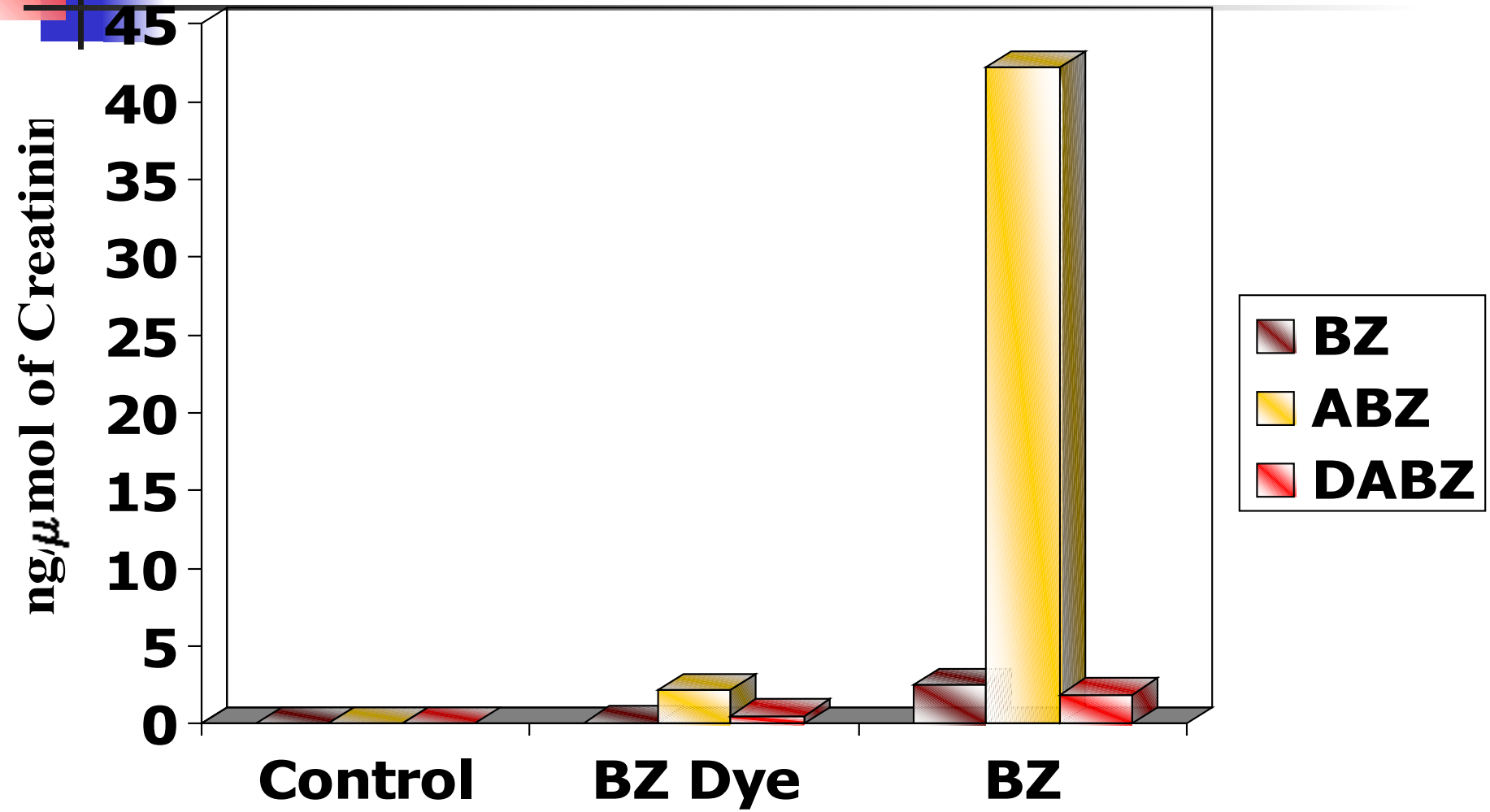
AUTORADIOGRAM of ^{32}P-DNA of OTHER WORKER involved in BZ production



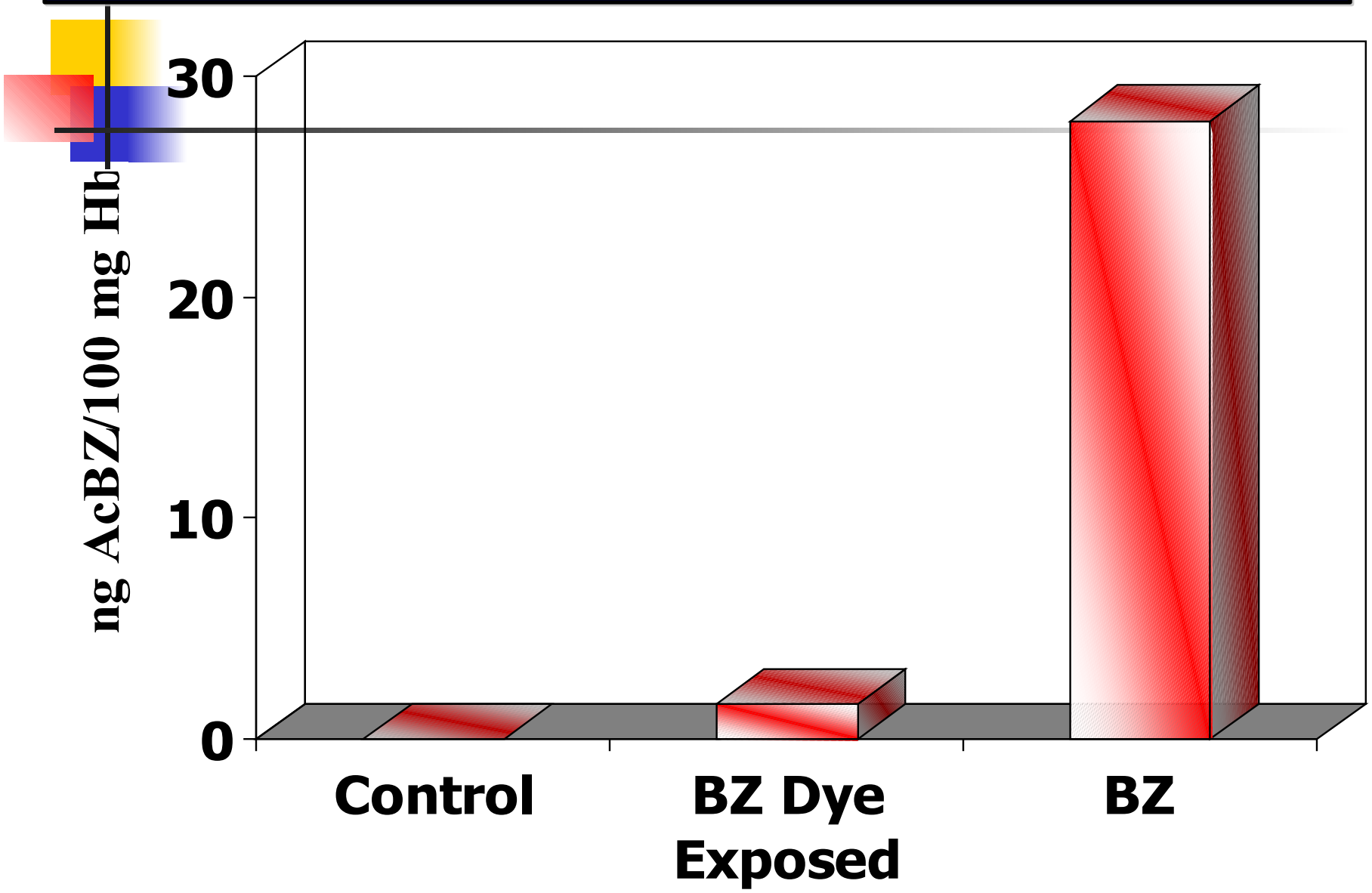
AUTORADIOGRAM of ^{32}P-DNA

of BZ WORKER

BZ metabolites in Control and Exposed Subjects



Hb-BZ adduct in Control and Exposed Subjects



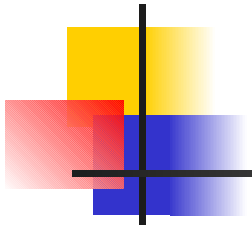
Conclusion

- Paolo Vineis
- ……finite
- Do's…….
- Right

Collaborators in Benzidine Project

N Rothman, G Talaska, T Zenser, D Bell, D DeMariani, V Lakshmi,

S K Kashyap, D Parikh, R Kashyap,



thanks