# Update on Health Research in Support of the Dioxin Reassessment

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# Biochemical Effects of TCDD

Enzyme Induction

- Cytochrome P4501A1
- Cytochrome P4501A2
- DT-Diaphorase
- UDP-Glucuronyl Transferase
- Glutathione-S-Transferase
- Aldehyde Dehydrogenase
- Ornithine Decarboxylase
- Tyrosine Kinase
- Thymidylate Tranferase
- Phosphoenólpyruvate Carboxykinase
- Plasminogen Activator Inhibitor-2

→ Altered Metabolism

# Effects of TCDD

- Death
- Wasting Syndrome
- Thymic Atrophy
- Splenic Atrophy
- Testicular Atrophy
- Liver Effects: Enlargement, Fatty Deposits, Necrosis
- Hyperplasia: Gastric Mucosa, Urinary Tract, Bile Duct
- Squamous Metaplasia: Meibomian Glands, Ceruminous Glands
- Chloracne: Hyperplasia, Hyperkeratosis, Altered Pigmentation
- Teratogenesis
- Carcinogenesis
- Immunosuppression
- Enzyme Induction
- Biochemical Effects

# Biochemical Effects of TCDD

Hormones and Receptors

- **Androgens**
- **Estrogens**
- **Estrogen Receptor**
- **Glucocorticoids**
- **Glucocorticoid Receptor**
- **Insulin**
- **Insulin Growth Factor**
- **Gastrin**
- **Thyroid Hormones**

→ Altered Homeostasis

# Biochemical Effects of TCDD

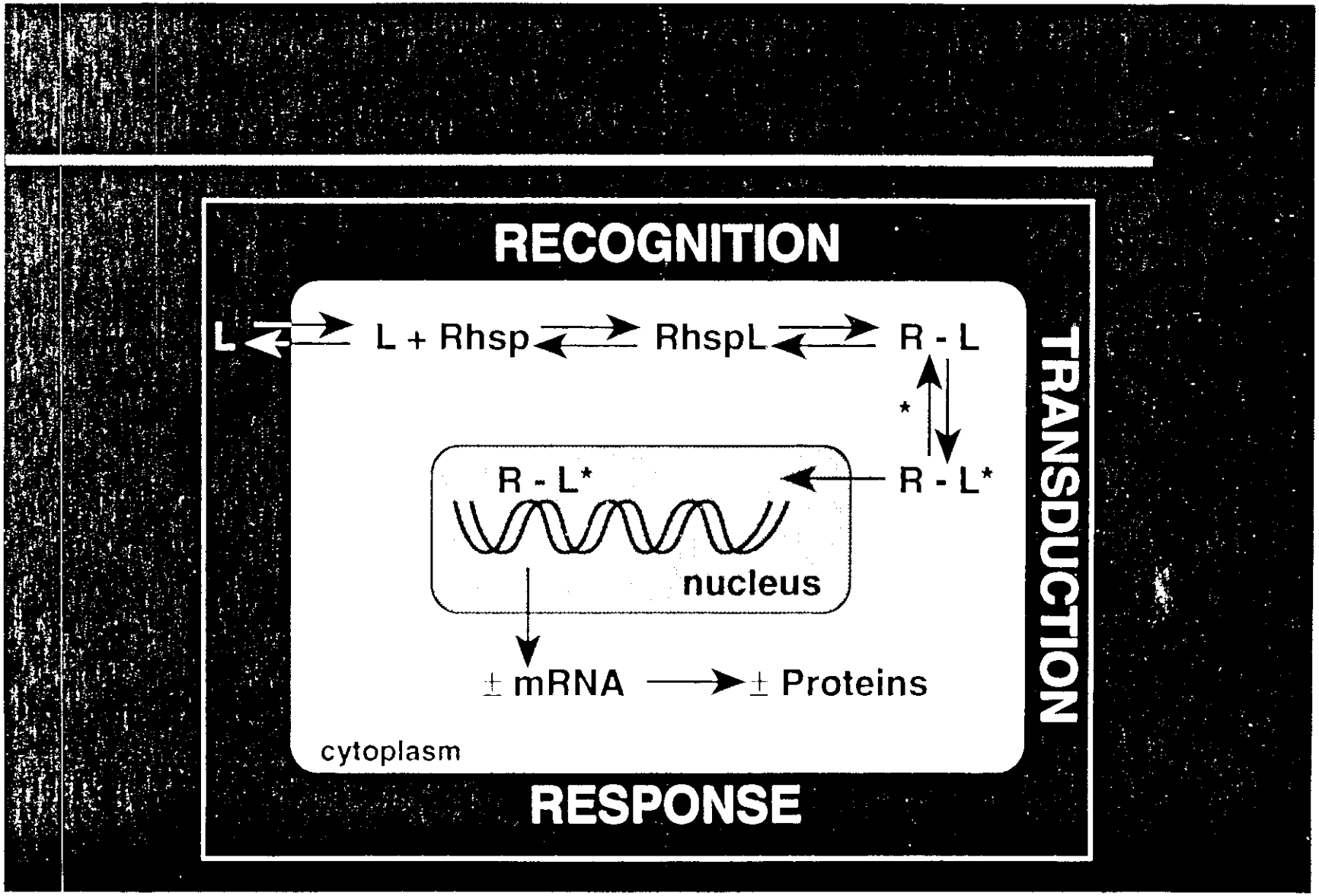
## Growth Factors and Receptors

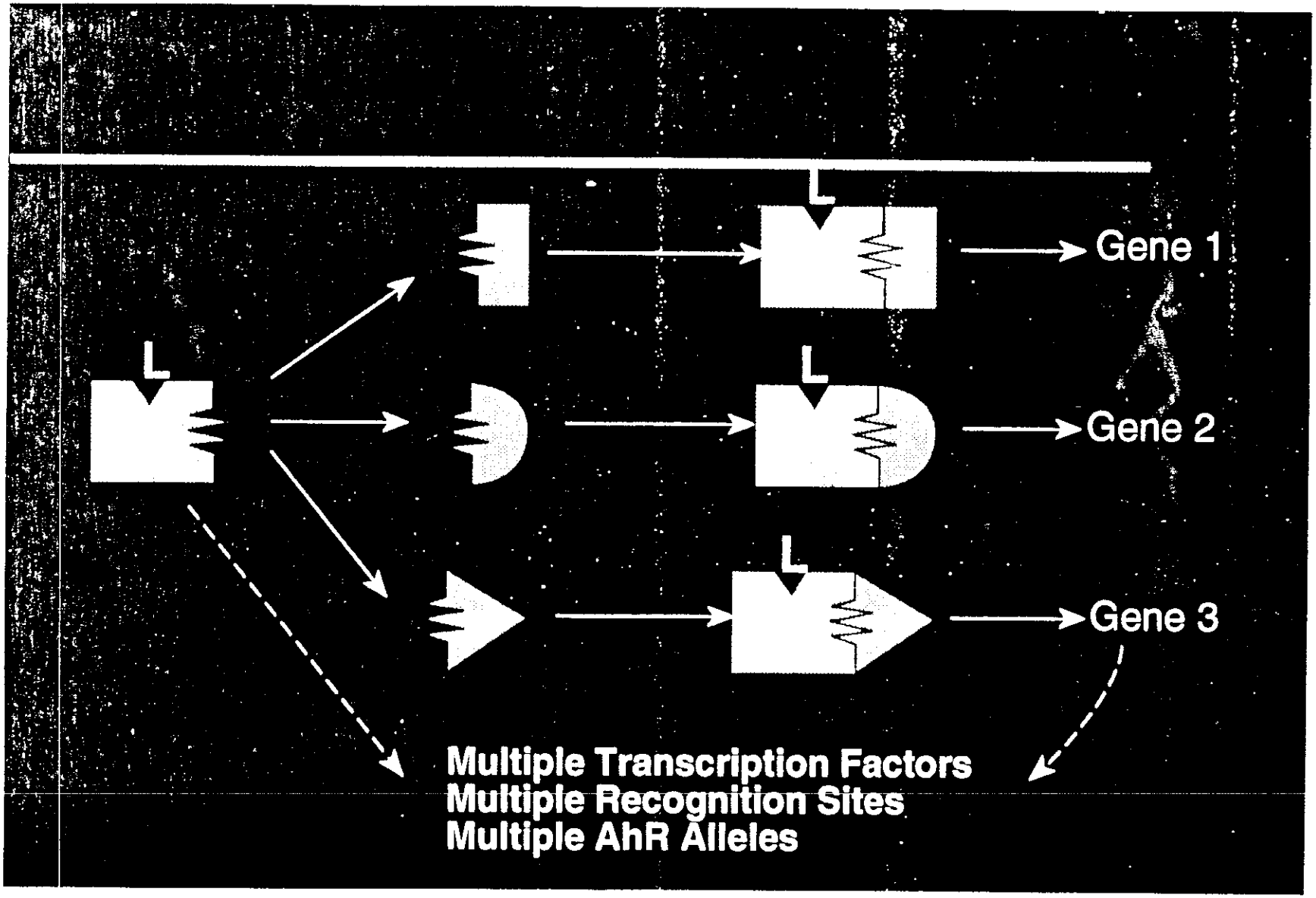
Vitamin A
EGF Receptor
TGFα , EGF

TGFβ1,β1
TNFα
IL1-β

Oncogenes
Ras
Myc
ErbA

→ Altered Growth and Differentiation


RECOGNITION
L
L + Rhsp
RhspL
R - L
*
R - L*
R - L*
nucleus
± mRNA
± Proteins
cytoplasm
TRANSDUCTION
RESPONSE

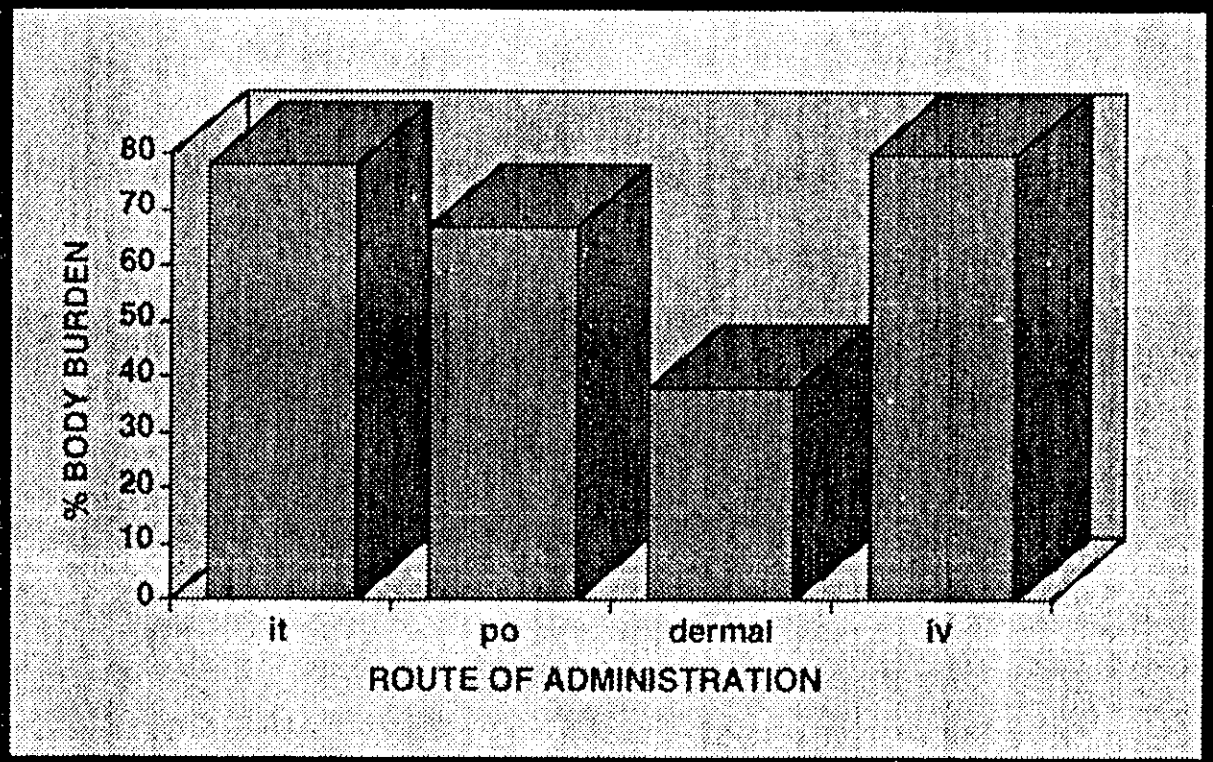

L
Gene 1
L
L
Gene 2
L
Gene 3
Multiple Transcription Factors
Multiple Recognition Sites
Multiple AhR Alleles

# Ah Receptor-New Understanding

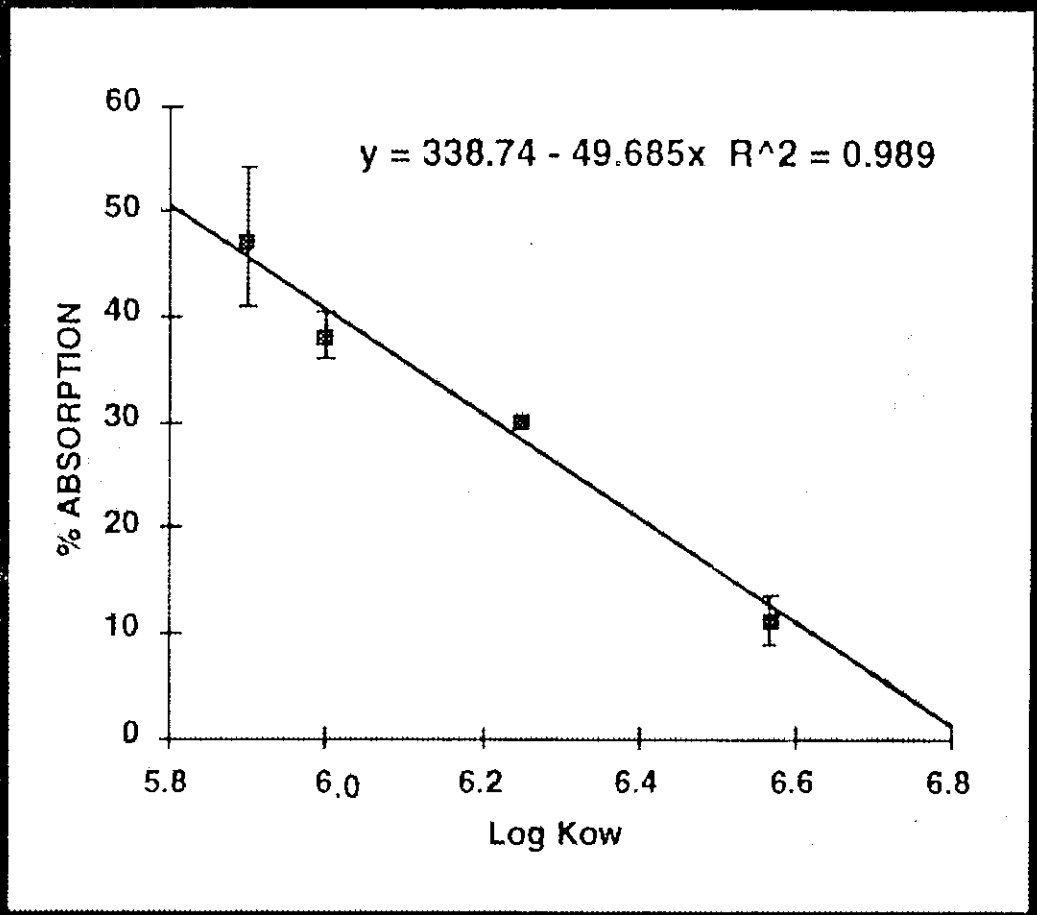
- The Ah receptor is similar (both qualitatively and quantitatively) in humans and experimental animals.
- Binding of TCDD and other ligands to the Ah receptor is controlled by interaction with other proteins. Specificity may be modified during development or in culture.
- Interaction of the Ah receptor with DNA requires additional proteins.

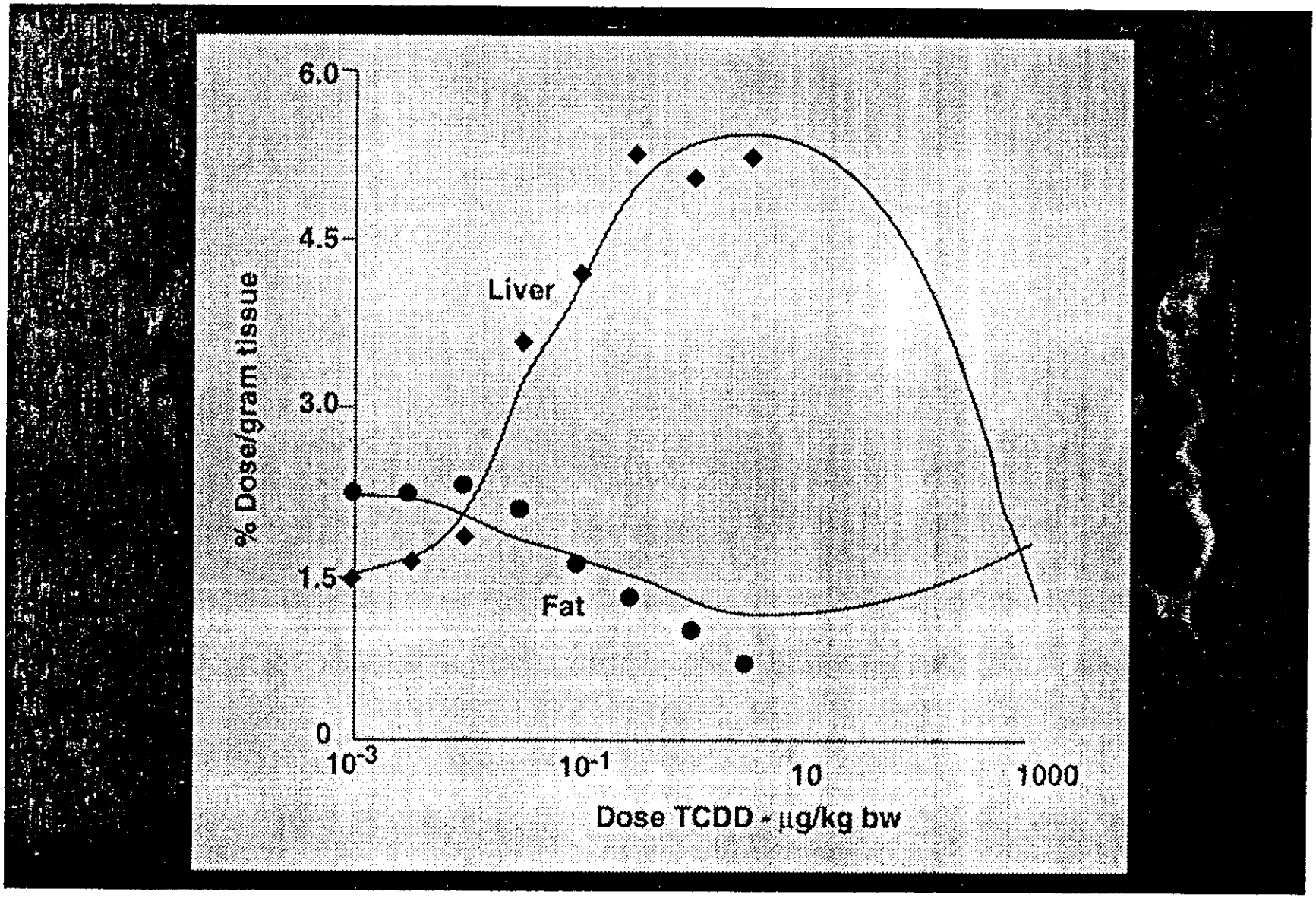
  Binding to the Ah receptor is necessary but NOT sufficient to bring about any biological response.

# % Body Burden 3 days after administration



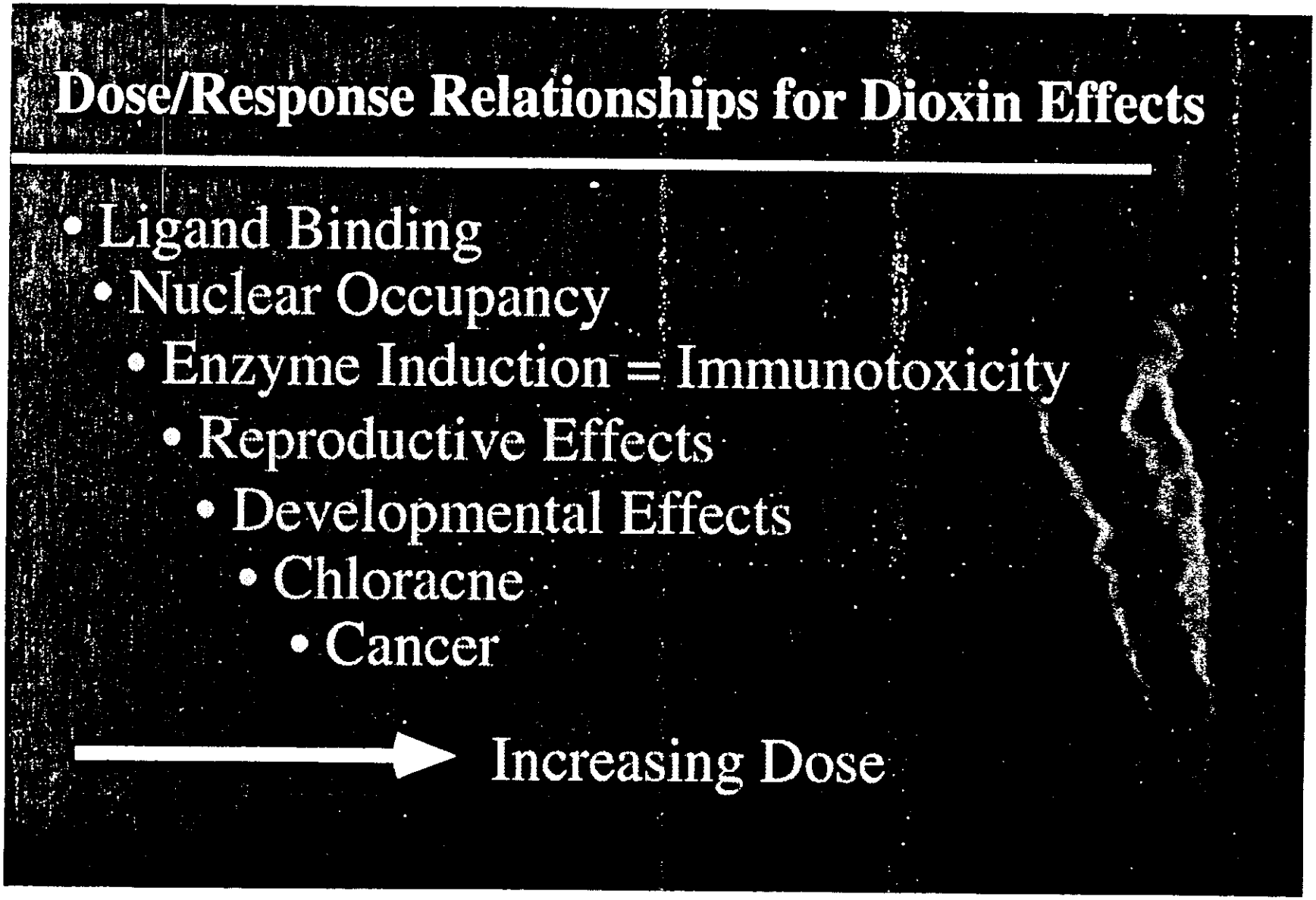
# Correlation of Dermal absorption of a 200 pmol DOSE with log Kow for TCDD, TCDF, TBDD, and TBDF




6.0
4.5
3.0
1.5
0
% Dose/gram tissue
Liver
Fat
$10^{-3}$
$10^{-1}$
10
1000
Dose TCDD - µg/kg bw

# Dosimetry - New Understanding

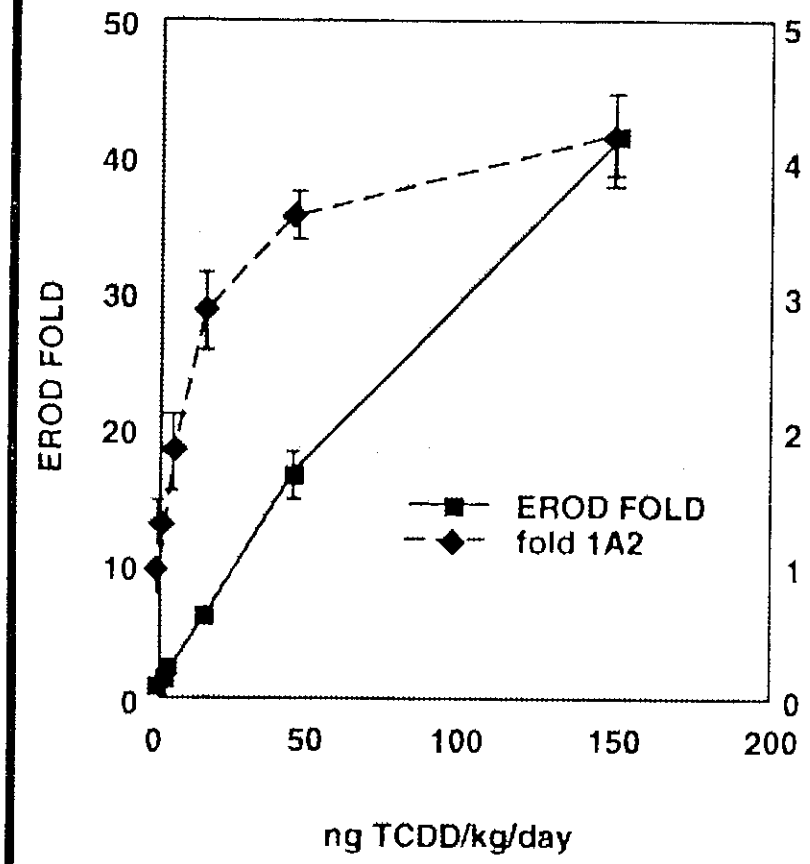
- Maximal absorption can occur via inhalation and ingestion. Dermal absorption is more limited.
- Dermal absorption can be predicted from the octanol-water coefficient.
- Tissue distribution is dose-dependent. At low doses, the relative amount to extrahepatic tissues will be greater than at higher body burdens.
- Physiologically-based pharmacokinetic models can be used to predict tissue distribution, and high to low dose and route-to-route extrapolations.

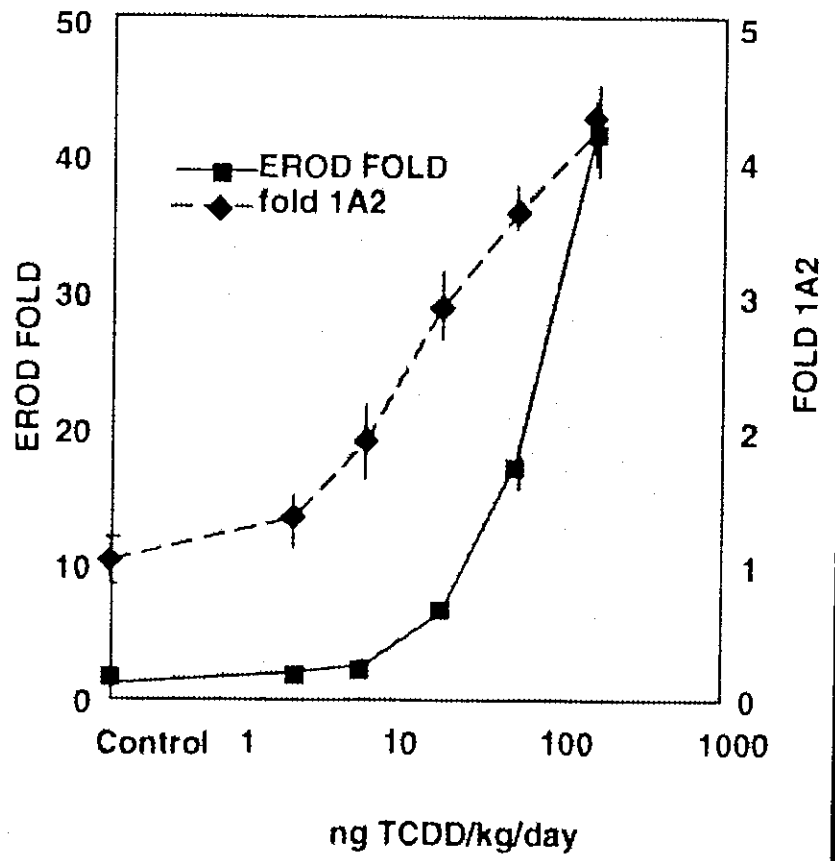

Dose/Response Relationships for Dioxin Effects
• Ligand Binding
• Nuclear Occupancy
• Enzyme Induction = Immunotoxicity
• Reproductive Effects
• Developmental Effects
• Chloracne
• Cancer
Increasing Dose

# Health Research in Direct Support of the Reassessment

- Comparative Sensitivity of Sensitive Endpoints
  - S. Safe, Texas A&M University
- Immunotoxicity
  - R. Smialowicz, HERL
  - R. Luebke, HERL
  - G. Burleson, HERL
- TEFs for PCBs
  - L. Birnbaum, HERL
- CYP1A1/1A2 in Human Liver
  - J. Goldstein, NIEHS

# Hepatic EROD and 1A2 from 90 day TCDD study



# Immunotoxicity - New Findings

- Suppression of Primary Antibody Response
  - In vivo: Very sensitive in mice and monkeys; Insensitive in rats
  - In vitro: Mice ≅ Humans
- Changes in T-Lymphocyte Populations
  - In vivo: Mice ≅ Marmosets
  - In vitro: Marmosets ≅ Humans
- Blockage of Host Defense Mechanisms
  - Influenza - Mice, Rats
  - Trichinella - Mice

# Relative sensitivity of different endpoints

- Enzyme induction≅Immunosuppression≅developmental/ reproductive toxicity
  Similar delivered dose results in response

- Alterations in gene expression -
  extremely sensitive measure of biological response, e.g., measurement of enzyme activity may be 10x less sensitive than measurement of mRNAs levels; use of PCR technology can detect changes in gene expression at 100x lower doses than necessary if enzyme activity is the endpoint

- Shapes of all dose/response curves are not the same

- Induction of certain responses appears linear at low doses

# Human Sensitivity

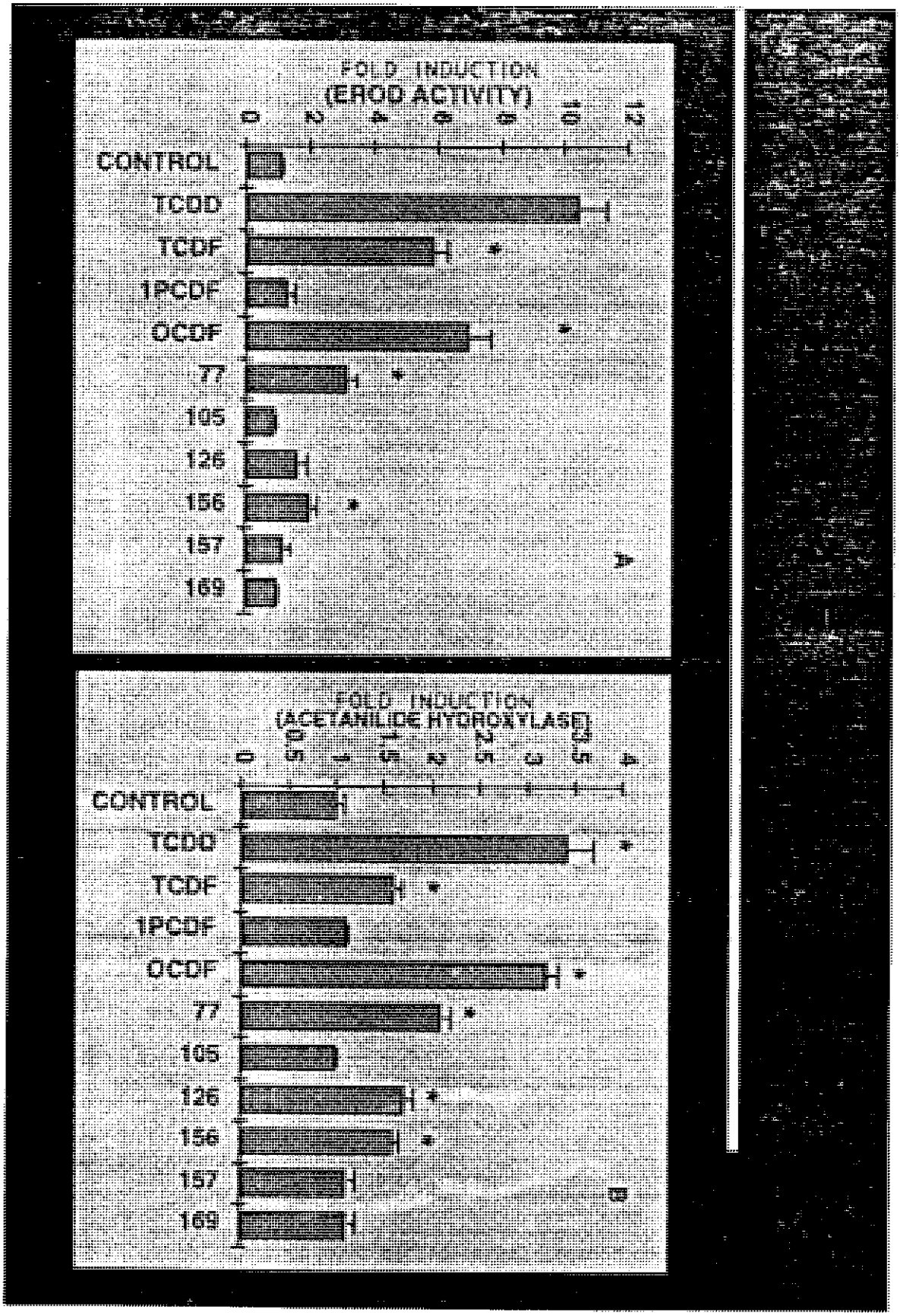
- Increases in CYP1A1/1A2 in Liver
  - 25 livers obtained
  - CYP1A1/1A2 measurements (mRNA and protein) completed
  - Samples awaiting congener-specific analysis (PCDDs, PCDFs, dioxin-like PCBs)

# What about PCBs?

- Only a small subset of 209 PCB congeners are dioxin-like
- Dioxin-like PCBs act through the Ah Receptor and induce the same spectrum of responses as TCDD
- The issue is not qualitative, but quantitative

# Relative Potency of Dioxin-like PCBs

- S. Safe (1990)
- Walker and Peterson, 1991
- Ongoing HERL Study
  - Female B6C3F1 Mice
  - 16 chemicals, 5 doses + control per chemical
  - Dosing 5 days/week for 13 weeks
  - Multiple Endpoints
    - Enzyme Induction
      - Liver: CYP1A1/1A2
      - Lung: CYP1A1
      - Skin: CYP1A1
    - Kinase Phosphorylation
    - Dosimetry


FOLD INDUCTION
(EROD ACTIVITY)
CONTROL
TCDD
TCDF
1PCDF
OCDF
77
105
126
156
157
169
FOLD INDUCTION
(ACETANILIDE HYDROXYLASE)
CONTROL
TCDD
TCDF
1PCDF
OCDF
77
105
126
156
157
169

# Preliminary TEF Results

- Enzyme Induction - Induction of CYP1A2 is a more sensitive response than CYP1A1
- Phosphorylation of a cell cycle control protein appears to be a more sensitive response than CYP1A1 induction
- The relative potency of the dioxin-like PCBs is much less (10-10,000 times) than that suggested by the conservative values of Safe (1990)

# Estimating Exposures to Dioxin-like Compounds

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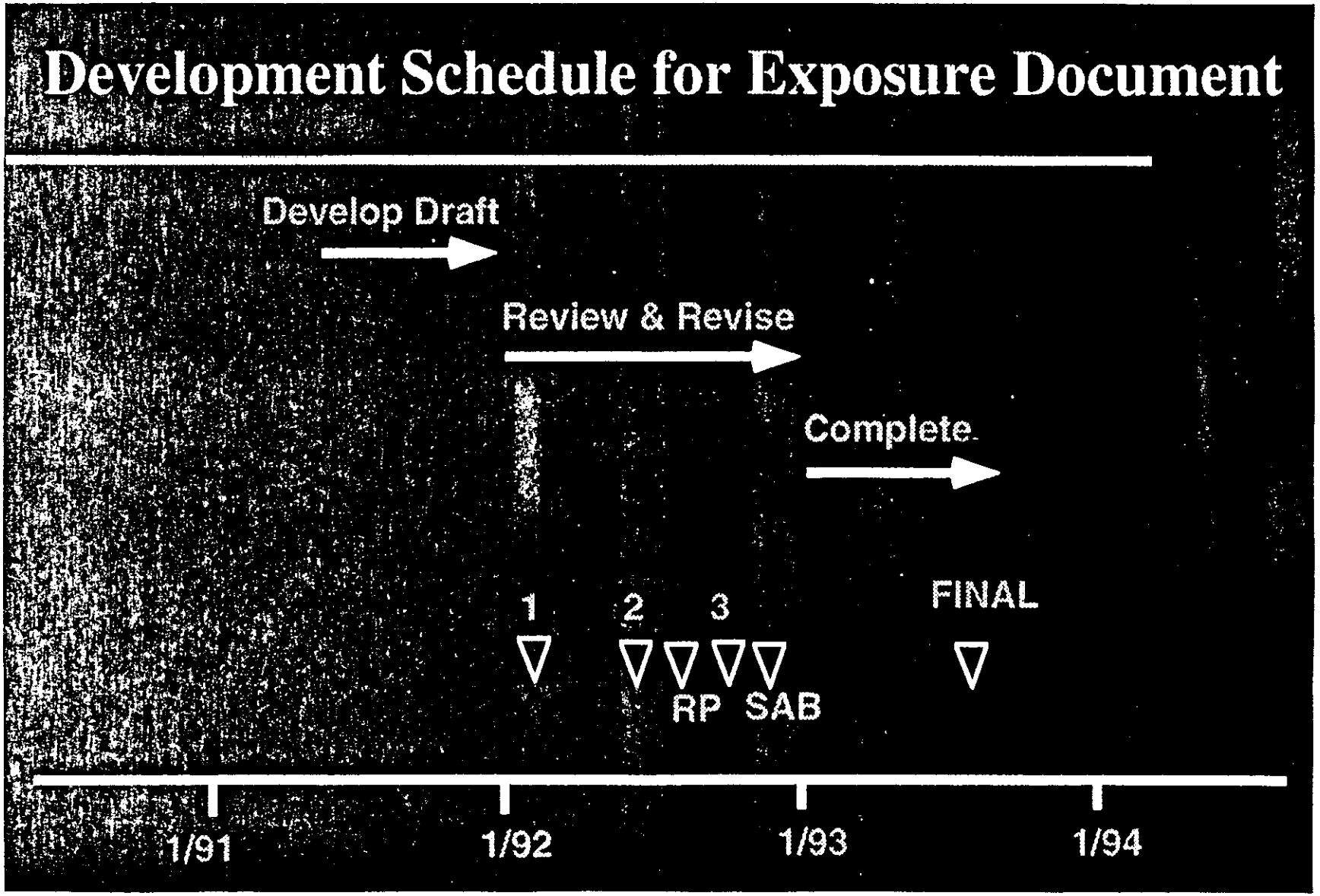
Office of Health and Environmental Assessment
Washington, DC

# Exposure Reassessment

- Purpose: Describe procedures for estimating exposure to dioxin - like compounds

- Complete 1988 draft "Estimating Exposure to 2,3,7,8-TCDD"


Development Schedule for Exposure Document
Develop Draft
Review & Revise
Complete
1
2
3
FINAL
RP
SAB
1/91
1/92
1/93
1/94

# Personnel Team

| Area | Primary Author | Lead Reviewer |
|---|---|---|
| Chem/Phys | Huse, G. Versar | Webster,G. U. Manitoba |
| Env. Levels | Huse,G. Versar<br>Lorber,M. EPA | Travis, C. Oak Ridge |
| Fate/Transport | Lorber,M. EPA | McKone, Lawrence Livermore |
| Exposure | Lorber,M. EPA | Falco,J. Battelle |
| Incineration | Cleverly,D. EPA | Tiernan,T. Wright State |
| Pharmacokinetics | Blancato,J. EPA | Olson, J. NY State U. |
| Uncertainty | White, P. EPA<br>Lorber, M. EPA | All |

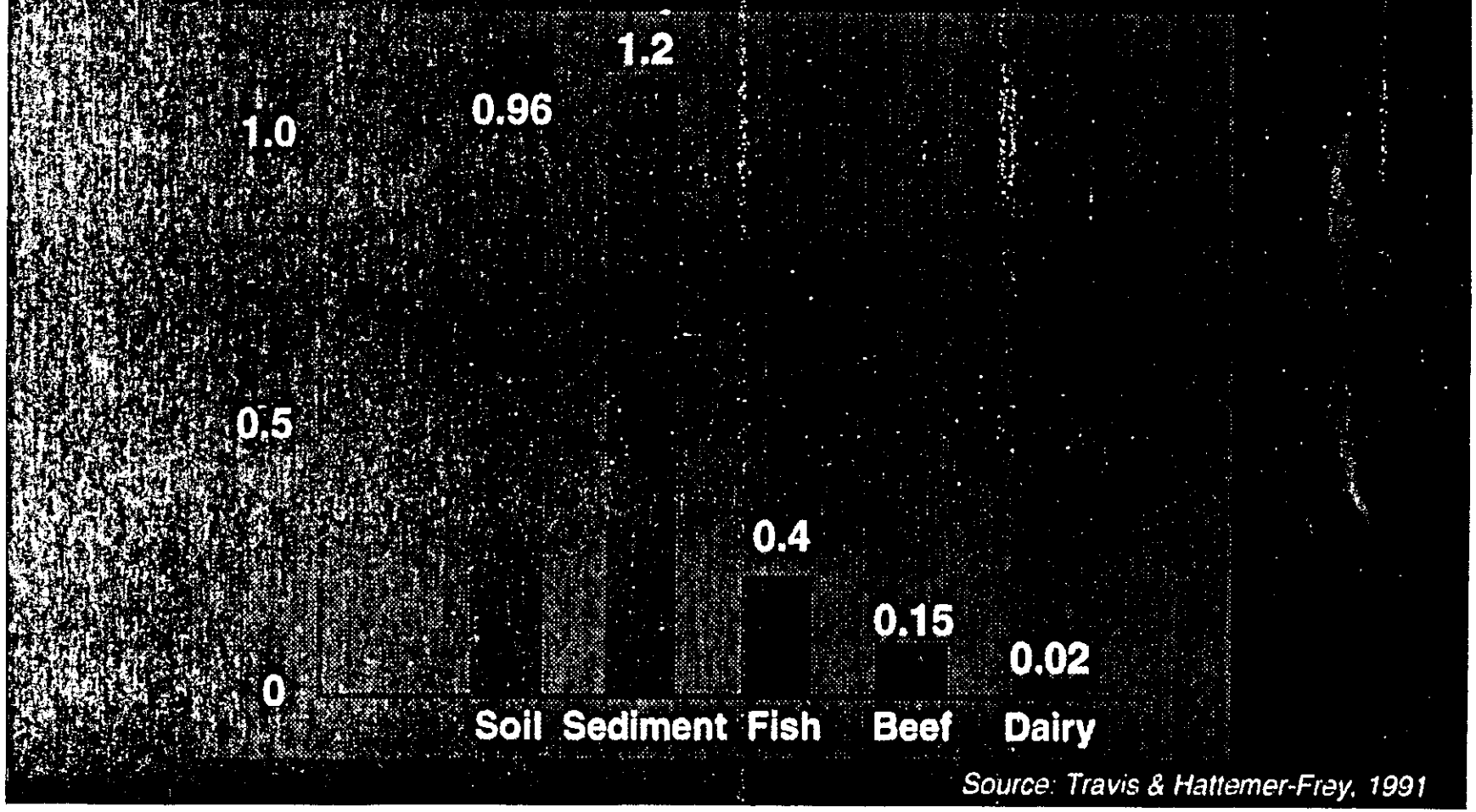
# Changes from 1988 Draft:

- Expansion to address all dioxin-like compounds
- Updating fate models
- Updating exposure parameters
- Updating pharmacokinetics
- Adding analysis of background exposures

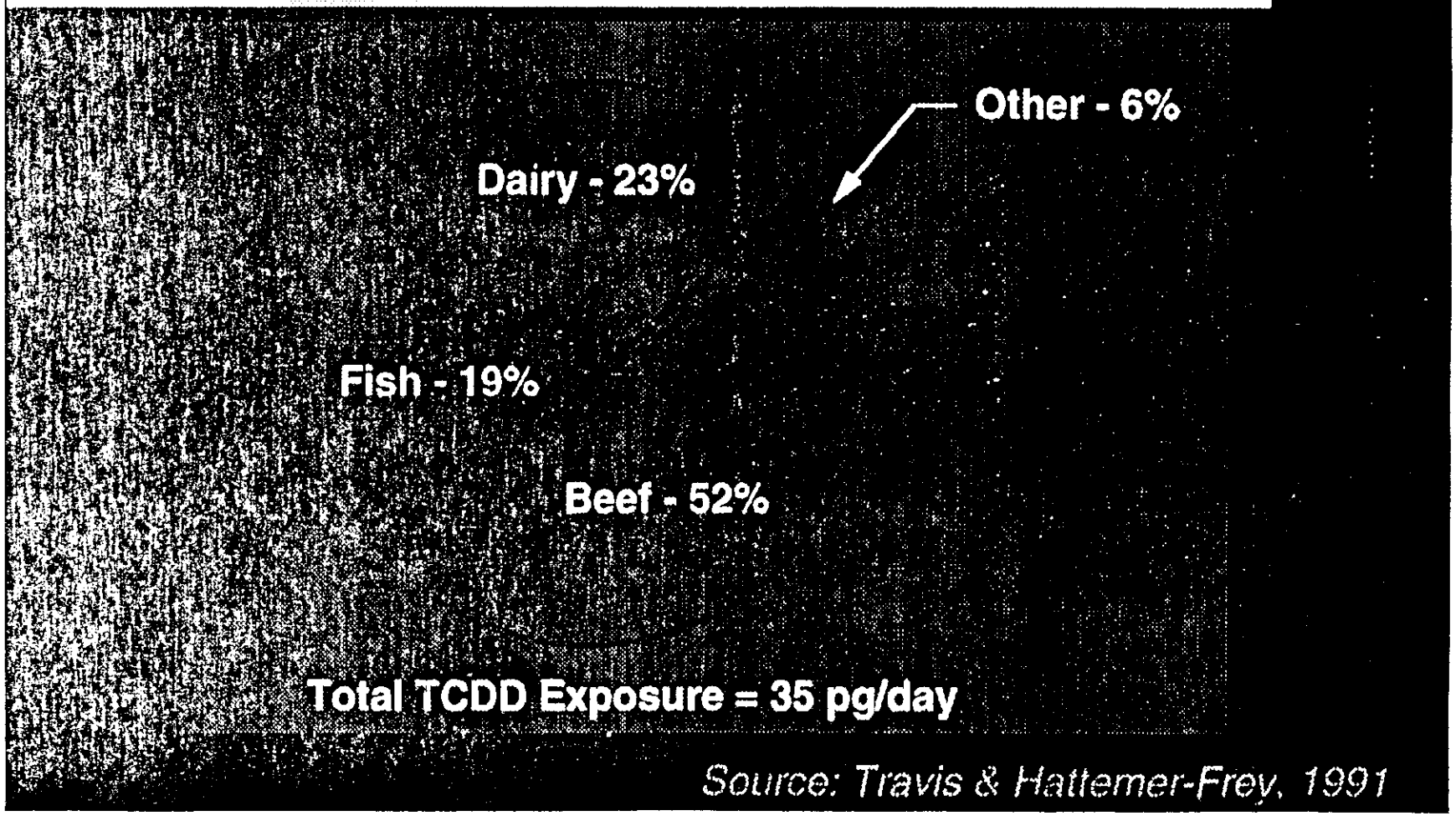
# Sources for Release of Dioxin to Environment

- Manufacture of chlorinated phenols, PCBs & phenoxy herbicides
- Bleached chlorine pulp and paper mill effluents
- Combustion processes burning chlorinated waste and fuels

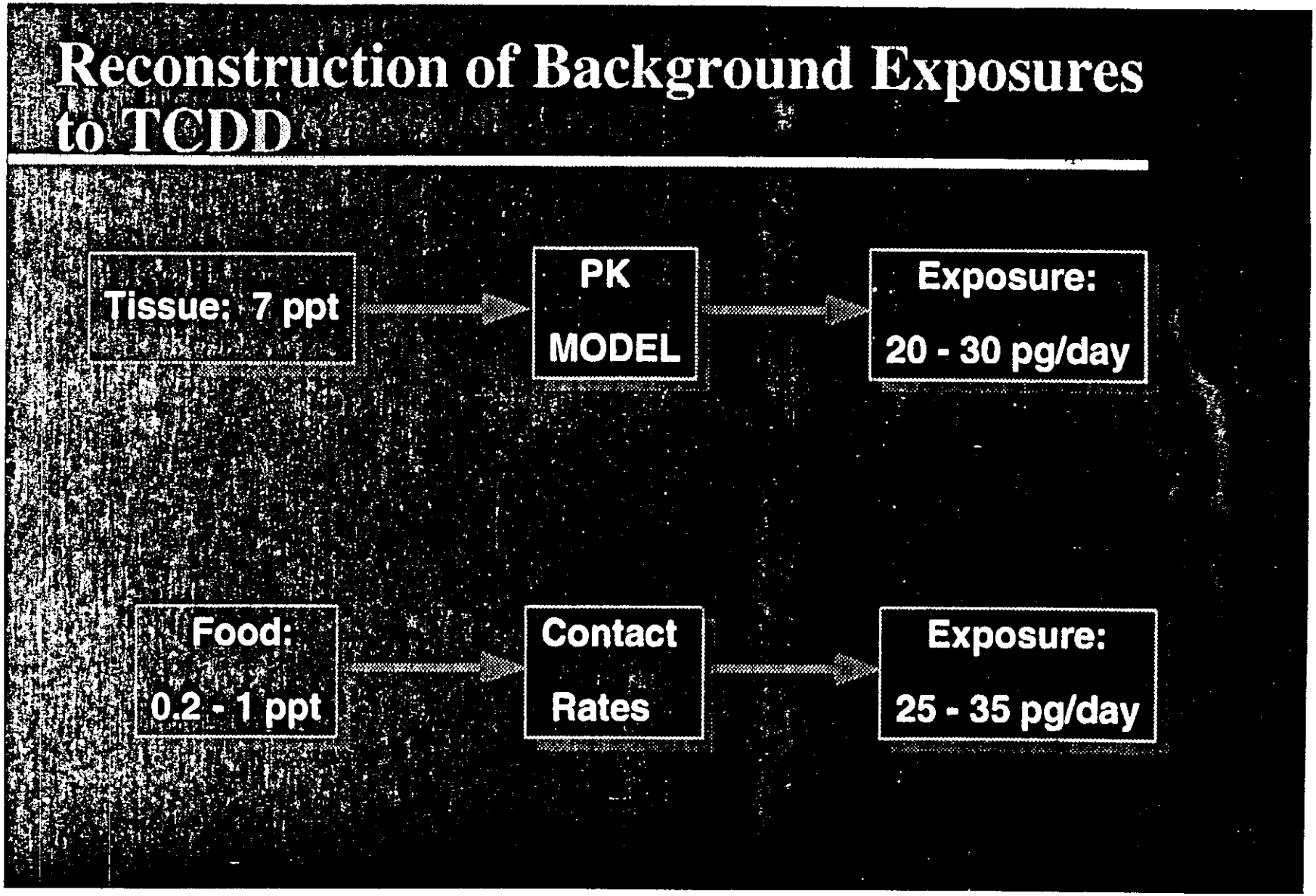
# Media Levels (NG TCDD/KG)



# Possible Background Exposures



# Reconstruction of Background Exposures to TCDD



# SITE - Specific Procedures

Sources:

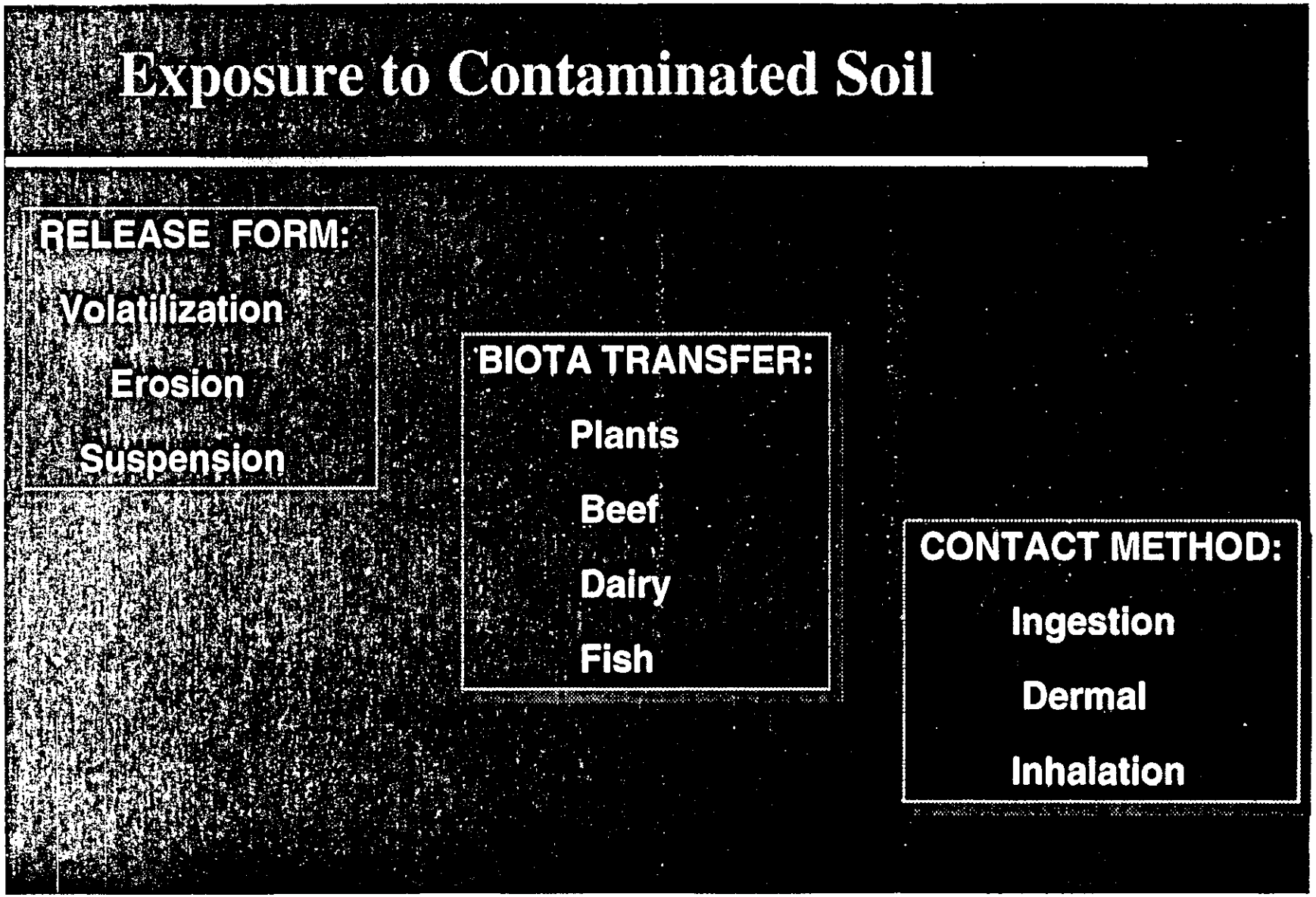
- Contaminated soil
- Incinerator emissions
- Effluent Discharges

Pathways:

- Ingestion of soil, vegetables, fruit, beef, dairy products, fish
- Inhalation of vapors and particulates
- Dermal contact with soils

# Exposure Assessment Process:

1. Identify Source
2. Estimate Release Rates
3. Estimate Exposure Point Concentration
4. Characterize Exposed Population
5. Define Exposure Scenarios
6. Estimate Exposure
7. Assess Uncertainty


Exposure to Contaminated Soil
RELEASE FORM:
Volatilization
Erosion
Suspension
BIOTA TRANSFER:
Plants
Beef
Dairy
Fish
CONTACT METHOD:
Ingestion
Dermal
Inhalation

# Dioxin Emissions from Incinerators

- New data on emission factors
- Expansion to all dioxin - like compounds

# Dioxin Emissions From Incinerators