Succinct Review

Principles

of

Toxicological Hazard and Risk Assessment

Professor Len Ritter
Risk Assessment

- Dose-response assessment
- Hazard identification
- Exposure assessment

Risk Management

- Risk characterization
- Risk management decisions
- Control options
- Legal considerations
- Other economic and social factors

Source: EPA Office of Research and Development
Adapted from NAS/NRC, 1983
Toxicological ADI
The toxicity of a xenobiotic is influenced by:

- Exposure (frequency, duration, intensity)
- Chemical characteristics
- Species of the test organism (strain, genetics)
- Age of the organism
- Sex of the organism
- Route of administration (or exposure)
- Nutritional status of the organism
- Disease or stress
- Other xenobiotics (synergy, additivity, antagonism)
DEFINITIONS

• Risk is a statistical expression of probability on a population basis (NOT individual); risk can be expressed as relative or absolute

• The probability of expression of a specified risk is a function of both the intrinsic toxicological properties (hazard) and the frequency, duration and intensity of exposure
DETERMINATION OF SAFETY

• RISK = HAZARD (TOXICITY) X EXPOSURE

• THEREFORE, AS EXPOSURE* DECREASES, SO DOES RISK

*Food+Air+Water+Occupational
DEFINITIONS

Toxicological outcomes can be

• Graded (continuously variable as in increasing hepatic fatty infiltration)
• Quantal (either “yes” or “no” without regard for severity, as in cancer induction or birth defects)
NON-THRESHOLD EFFECTS

- Effects that occur at any exposure level in some individuals of a population
- Statistical power to detect non threshold effects
- Mostly genotoxic carcinogens, but true for other effects, too (e.g. lead neurodevelopmental toxicity)
THRESHOLD EFFECTS

- Effects that are not observed (do not occur?) below a specified level of exposure
- Distribution of doses may influence interpretation
Stylized curves for some representative dose-response relations:
(A) the threshold model, (B) the linear, nonthreshold model, (C) the inverted U-shaped hormetic model depicting low-dose enhancement and high-dose reduction of normal function effects, and (D) the J-shaped hormetic model depicting low-dose reduction and high-dose enhancement of adverse dysfunction effects.
tRISK ASSESSMENT PARADIGM

- Hazard assessment
- Dose-response assessment
- Exposure assessment
- Risk characterization
"The dose that makes the poison."

Paracelsus, 1493-1591
Theophrastus Philippus Aureolus Bombastus von Hohenheim
TOXICOLOGICAL STUDIES (Pre-Clinical)

- Acute
- Eye and Skin Irritancy
- Sub-Chronic
- Chronic
- Carcinogenicity
- Mutagenicity and Genotoxicity
- Reproductive
- Teratology (Developmental)
- Delayed Neurotoxicity
- Exposure
All of these studies are characterized by very exaggerated exposure (dose) levels which may be thousands to tens of thousands of times greater than expected human exposure—**the challenge in toxicology is to relate the effects seen at the high experimental levels to the more realistic levels to which humans are typically exposed.**
Exposure(dose)-Response Relationship

Response

Exposure

General Population

Epidemiological Studies

Animal Studies
Approximate Number of Animals Required for 95% Confidence Interval Equal to Size of the Effect (Spontaneous Background Rate = 0.01)

<table>
<thead>
<tr>
<th>Case</th>
<th>Treated</th>
<th>Prop. of Animals with tumors control</th>
<th>Excess (C.I.) 95%</th>
<th>Approximate sample size (each)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED.001</td>
<td>.011</td>
<td>.01</td>
<td>.001</td>
<td>+/- .001</td>
</tr>
<tr>
<td>ED 1</td>
<td>.02</td>
<td>.01</td>
<td>.01</td>
<td>+/- .01</td>
</tr>
<tr>
<td>ED 10</td>
<td>.11</td>
<td>.01</td>
<td>.10</td>
<td>+/- .10</td>
</tr>
</tbody>
</table>
CARCINOGENS ARE CHARACTERIZED BY A “NO-THRESHOLD” DOSE RESPONSE RELATIONSHIP – THERE IS SOME LEVEL OF RISK, EVEN IF IT CANNOT BE DIRECTLY MEASURED, AT EVERY LEVEL OF EXPOSURE.
Virtually Safe Dose
Virtually Safe Dose

Acceptable Increment in Background Response

Probability of Response

Virtually Safe Dose

Dose
MAJOR ASSUMPTIONS IN RISK ASSESSMENT

- Dose-response data from limited size groups of laboratory experimental animals, of homogenous genetic make-up, can predict the dose-response relationship for a very heterogeneous human population.
- Dose-response data obtained at high levels of exposure relative to expected environmental exposures can predict the dose-response relationship at these lower doses which are usually well below the experimental range.
- Dose-response data obtained from consistent experimental dosing regimens can predict the dose-response relationships for much more variable human exposures.
Non Carcinogen (threshold) Assessment

There is some level of exposure at which there is no toxicologically relevant response (acute or chronic)
NO(A)EL

• The highest dose* administered to a population of test animals which does not produce evidence of an adverse effect

* as most chemicals are tested in a wide array of toxicological studies, the NO(A)EL is selected from the most sensitive, yet appropriate endpoint
“the daily intake of a chemical which, during an entire lifetime, appears to be without appreciable risk on the basis of all facts known at the time. Without appreciable risk is taken to mean the practical certainty that injury will not result even during a lifetime of exposure”

Lu and Sielken, 1991
tADI

\[
\text{ADI} = \frac{\text{NOAEL (BMD)}}{\text{Safety (Uncertainty) Factor}} = \frac{R_D}{D} \text{ (reference dose)}
\]
The use of uncertainty or safety factors are used to extrapolate from a group of test animals to an average human and from average humans to potentially sensitive sub-populations. Up to an additional 10x to protect children.
Principles for the Assessment of Risks to Human Health from Exposure to Chemicals, EHC 210, 1999; EHC 240
US EPA Guidelines for Uncertainty Factors

Guideline | Factors
----------|----------
Average   | Sensitive human ≤10x
Animal    | Human ≤10x
LOAEL     | NOAEL ≤10x
Database Inadequacies | ≤10x
Subchronic | Chronic ≤10x
Modifying factors (FQPA) | ≤10x

Faustman, 1997
Acute Reference Dose - ARD

Acute Reference Dose (ARD):

- the amount that can be ingested orally on any given day without appreciable risk

\[
\text{NOAEL (mg/kg bw)} \quad = \quad \text{ARD} \\
\text{Uncertainty Factor (100x minimum)}
\]
Reference Dose Determination

Identify Acute Reference Dose (ARD)
- amount of pesticide /drug considered safe for human consumption on any one day

Identify Chronic Acceptable Daily Intake (tADI/RfD)
- amount of pesticide /drug considered safe for human consumption each day for a lifetime
Reference Dose - tADI

Acceptable Dietary Daily Intake (ADI):

- the amount that can be ingested daily (orally) over a lifetime without appreciable risk

NOAEL (mg/kg bw/day) = tADI
Uncertainty Factor (100x minimum)
Margin of Safety

\[
\text{HBGV} \\
\text{Exposure}
\]

Margin of Exposure

\[
\text{NOAEL} \\
\text{Exposure}
\]
EXPOSURE ASSESSMENT
Food Residue Evaluation

OBJECTIVES

Quantify maximum residue limits
- on crops, and in animal-derived food products (i.e., meat, milk, eggs) at the point of sale

Define residue(s) of concern
- Parent plus any metabolite, degradation product or impurity that may be of toxicological concern
Propose Maximum Residue Limits (MRLs)
- Maximum amount of pesticide/drug likely to be in or on food when used in accordance with GAP (good agricultural practice) - i.e., at label rates

Estimate potential dietary intake (PDI)
- Total from food sources and water for general pop., sensitive sub-populations - infants, children
- PDI must not exceed reference doses (ARD/ADI)
Food Residue Data

Label
- use pattern - rates, PHI/withdrawal times and crops/livestock treated

Product chemistry
- specifications, impurities, physical and chemical properties

Analytical methods
- for determining residues in food (precision, accuracy)
Risk Management Food Residues

**Risk Management Trigger:** PDI greater than RfD

**Options:**

- Remove select crops/tissues from label
- No treated crops as animal feed
- Increase post-treatment intervals
- Obtain definitive residue data for crops/tissues based on actual use (use not always maximum label rates or frequency)
- Reduce label rates/doses
- Reduce application/treatment frequency
- Discontinue registration
Uncertainty in the default assumptions used to predict safety of residues in food............

- All of the crop/species are treated all of the time at maximum rate
- Only treated food is consumed
- Residues are always present at the maximum level permitted by regulation
- Humans are more sensitive than the most sensitive animal species used in the toxicology studies
Microbiological ADI
• Risk assessment, data requirements, and test methods for safety evaluation of veterinary drugs in food: Impact on intestinal microbiota
• Public health and regulatory issues
• Determination of no effect levels (NOAEC) for disruption of colonization barrier and antimicrobial resistance
  ✓ Traditional and current molecular approaches to determine impact of veterinary drug residues on the human intestinal microbiota.
• Recent research on determining fraction of oral drug dose available to human intestinal microbiota
  ✓ Fecal binding, fecal drug inactivation and metabolism studies
The total number of these bacterial cells is estimated to be more than $10^{14}$, accounting for 10 times more than the total number of eukaryotic cells that compose a human individual.

Our human body sites are colonized by an enormous number of microorganisms, of which the majority is bacterial species, and they form complex communities called the human microbiota.

The total number of these bacterial cells is estimated to be more than $10^{14}$, accounting for 10 times more than the total number of eukaryotic cells that compose a human individual.

Among them, the gut microbiota is the largest and most complex, and is composed of more than 1,000 different intestinal microbes.

<table>
<thead>
<tr>
<th>Body Site</th>
<th>Bacterial/ml or gram</th>
<th># of species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nose</td>
<td>$10^3 - 10^4$</td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td>$10^{10}$</td>
<td>&gt;700</td>
</tr>
<tr>
<td>Saliva</td>
<td>$10^8 - 10^{10}$</td>
<td></td>
</tr>
<tr>
<td>Gingival crevice</td>
<td>$10^{12}$</td>
<td></td>
</tr>
<tr>
<td>Tooth surface</td>
<td>$10^{11}$</td>
<td></td>
</tr>
<tr>
<td>GI tract</td>
<td>$10^{14}$</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Stomach</td>
<td>$10^0 - 10^4$</td>
<td></td>
</tr>
<tr>
<td>Small intestines</td>
<td>$10^4 - 10^7$</td>
<td></td>
</tr>
<tr>
<td>Colon (feces)</td>
<td>$10^{11} - 10^{12}$</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>$10^{12}$</td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>$10^5$</td>
<td></td>
</tr>
<tr>
<td>Urogenital</td>
<td>$10^{12}$</td>
<td></td>
</tr>
<tr>
<td>Vagina</td>
<td>$10^9$</td>
<td></td>
</tr>
<tr>
<td>Human cells</td>
<td>$10^{13}$</td>
<td></td>
</tr>
</tbody>
</table>
Meat producers have fed growth-promoting antibiotics to food animals for years.

Recently, scientists have raised concerns that, in conjunction with the general overuse of antibiotics in humans, this use of "sub-therapeutic" levels of antibiotics in food animals may lead to serious health risks for people.

Animal Health Industry Perspective

- Banning the use of such drugs, however, would greatly reduce the efficiency of the industry, driving up the cost of meat.
- Some in the industry believe that the scientific evidence linking low-dose usage of antibiotics to drug-resistant illnesses in people is too inconclusive and does not justify banning their use.
Concerns Associated with Effects of Antimicrobial Drug Residues on Human Intestinal Microbiome are Founded on the Assumptions:

a) Regardless of route of administration, antimicrobials can reach the colon due to incomplete absorption or by recirculation via the bile of secretion across the intestinal mucosa.

b) By virtue of their inherent antimicrobial activity, these free (active) drug concentrations are likely to inhibit at least part of the rich microbiota of the gastrointestinal tract.
Public Health/Regulatory Questions Raised

• What amount of antimicrobial substance is available to the bacteria in the lower part of the gastrointestinal tract?
• Does the use of veterinary drugs in food animals increase the spread of antibiotic resistance?
• What concentration of an antimicrobial residue would have a no effect on human intestinal microbiota?
• What experimental *in vitro* and *in vivo* approaches should be used to measure potential microbiological effects?

**Regulatory problem**: The impact of residual levels of veterinary drugs in food on the bacteria and selection of resistant bacteria in the gastrointestinal tract are not fully understood
It should be noted that antimicrobial residues in food are a different issue from resistant organisms acquired from food or other persons.

However, it is potentially related to the issue of antimicrobial selection pressure that could play a role in transmission and carriage of resistant intestinal commensal species to and among humans.
Antimicrobial residues are evaluated internationally by:

- Food and Drug Administration (FDA)
- Joint Expert Committee on Food Additives (JECFA)
- European Medicines Evaluation Agency (EMA/CVMP)
- Veterinary International Cooperation on Harmonization (VICH)
- Codex Alimentarius Commission (CAC)
- Other National Regulatory Authorities (Health Canada)
Guidance for Industry
Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food:
General Approach to Establish a Microbiological ADI
VICH GL-36

FINAL GUIDANCE

(This guidance replaces CVM’s Guidance for Industry # 52 entitled “Assessment of the Effects of Antimicrobial Drug Residues from Food of Animal Origin on the Human Intestinal Flora” dated February 18, 2004.)

Comments and suggestions regarding the document should be submitted to Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to http://www.fda.gov/dockets/ecomments. All comments should be identified with the Docket No. 2003D-0474.

For questions regarding this document, contact Louis T. Mulligan, Center for Veterinary Medicine, (HFV-153), Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855, 301-827-6984, e-mail: lmulliga@cvm.fda.gov.

Additional copies of this guidance document may be requested from the Communications Staff (HFV-12), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at http://www.fda.gov/cvm.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
February 10, 2005
Step 1. Are residues of the drug/metabolites microbiologically active against human intestinal flora?

- Yes
- No

Step 2. Do residues enter the human colon?

- No
- Yes

Step 3. Do the residues entering the human colon remain microbiologically active?

- No
- Yes

Step 4. Assess scientific justification to eliminate testing either one or both endpoints

- No
- Yes

Step 5. Determine the NOAEC/NOAEL for the endpoint of concern (step 4). The most appropriate NOAEC/NOAEL is used to determine the mADI.

No concern of drug residue effect on human gut microbiota. Toxicological ADI is applied.

mADI versus tADI: the smaller one will be the final ADI
**MICcalc:**
The MICcalc is derived from the lower 90% confidence limit for the mean MIC50 of the relevant genera for which the drug is active, as described in Appendix C of VICH GL36.

**NOAEC:**
The NOAEC derived from the lower 90% confidence limit for the mean NOAEC from *in vitro* systems should be used to account for the variability of the data.

**Mass of colon content:**
The 220 g value is based on the colon content measured from accident victims.

**FAM(Fraction of an oral dose available for microorganisms):**
It is recommended that the fraction of an oral dose available for colonic microorganisms be based on *in vivo* measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine.

**Body weight:** 60 kg

\[
ADI = \text{MICcalc} \times (\text{NOAEC}) \times \text{Mass of Colon Content (220 g)} \times \text{FAM} \times 60 \text{ kg person}
\]
Antimicrobial Resistance

(the VICH Guidance #36)

Antimicrobial Resistance:

increase of the population(s) of bacteria in the intestinal tract that is insensitive to the test drug or other antimicrobial drugs. Effect may be due either to acquisition of resistance by organisms previously sensitive or to relative increase in proportion of organisms already less sensitive to the drug.
We have more bacteria in our body than there are people living on earth.

- **Colon**
  - Bacterial numbers: c.a. $10^{12}$/g contents

- **Stomach**
  - Bacterial numbers: c.a. $10^3$/ml contents

- **Small Intestine**
  - Bacterial numbers: c.a. $10^4 - 10^5$/ml contents

Typical adult body has 100 trillion bacterial cells consisting of at least 800 species in the GI tract. 100X more genes in the gut microbiome than in the 2.85 billion bp human genome.
### Current Use of Antimicrobials in Animals

<table>
<thead>
<tr>
<th>Productions Animals</th>
<th>Companion Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapeutic</td>
<td>Therapeutic</td>
</tr>
<tr>
<td>Prophylactic</td>
<td>Prophylactic</td>
</tr>
<tr>
<td>Sub-Therapeutic</td>
<td></td>
</tr>
</tbody>
</table>

- Animal health uses 60% to 80% of all produced antimicrobials
- In the US, 33.33% used to enhance production (includes the ionophores)
- 18-25 million pounds used sub-therapeutically (for growth promotion): banned in the European Union since 2006

Concern: Same classes as those used in human medicine
Meat producers have fed growth-promoting antibiotics to food animals for years.

Recently, scientists have raised concerns that, in conjunction with the general overuse of antibiotics in humans, this use of "sub-therapeutic" levels of antibiotics in food animals may lead to serious health risks for people.

Banning the use of such drugs, however, would greatly reduce the efficiency of the industry, driving up the cost of meat.

Some in the industry believe that the scientific evidence linking low-dose usage of antibiotics to drug-resistant illnesses in people is too inconclusive and does not justify banning their use.
Human Food Safety Assessment of Veterinary Drugs

Toxicity Assessment

Lab Animal Toxicity Data

No Observed Adverse Effect Level (NOAEL)

Toxicological or pharmacological ADI

Microbiological Safety Assessment

AMR / Effect on Gut Microbiota

Microbiological ADI

Metabolism & Residue Chemistry Assessment

Establishment of MRLs and WP

Lowest ADI
Food Safety Perspectives

- **Toxicology:**
  - Evaluates battery of tests to identify systemic acute and chronic toxicity, genotoxicity (mutagenicity), reproductive/developmental toxicity, and
  - NO(A)EL → ADI → Safe concentration in edible tissues

- **Residue Chemistry:**
  - Evaluates drug metabolism and depletion studies
  - Identifies marker residue and target tissue
  - Determines Tolerance and setting Withdrawal period

- **Microbial Food Safety:**
  - Addresses antimicrobial resistance issues: risk compromising human medicine (GFI #152)
  - Addresses microbiological effects on human intestinal bacteria – mADI [GFI #159 (VICH GL36)]
First, not all food-producing animals are treated with antimicrobials, and of these few will have tissue residues at the MRL.

Second, *in vivo* adsorption, chemical or bioinactivation via metabolism and dilution of antimicrobial residues in the human gut may further lower the concentration of antimicrobials in the lumen of the gastrointestinal tract that is available to come in contact with the intestinal microbiota.
Gathering Evidence To Determine Whether Or Not Antibiotic Residues In Food Can Modify The Antimicrobial Resistance Profile Of Human Gut Microbiota Is Problematic

- Third, the degradation of residues associated with food processing and cooking may result in lower concentrations of microbiologically active residues in the prepared food.
- Fourth, distribution of microbiota in the human bowel in relation to antibiotic residues.
Antimicrobial residues in foods makeup a small fraction of total antimicrobials to which persons are exposed to in terms of either frequency or dose.

Humans are never exposed continuously to antimicrobial residues and the food of animal origin will be diluted with the total mass of food and fluid ingested.

The food commodities in which the residue is present may not be part of the daily diet of the consumer or may not be present in the edible portion of the commodity.

Drug residues are so low due to the conservative nature of the assignment of ADIs, MRLs, withdrawal times, and since not all animals are treated nor slaughtered at legally established withdrawal times.
Microbiological ADI Determination - Chronology

• **1960s:** Introduction of ADI concept in EU and JECFA

• **1990s:** JECFA’s guidance on the data requirements and the decision tree approach for mADI determination (1999)
  - Determination of the most sensitive adverse effects on the human intestinal Microbiota with three concerns:
    - Emergence of antimicrobial resistant population
    - Disruption of the colonization barrier
    - Changes in enzymatic activities (NOEL determination)

• **2000s:** Development and Implementation of VICH GL-36 (2004):
  - Two endpoints and 5 steps
    - JECFA harmonized with VICH GL-36 (2006)

• **2012:** Implementation of revised VICH GL-36 with new guidance on test systems and assay methodology for assessment of the fraction of oral dose available to microorganisms in the intestine
Step 1: Are residues of drug/drug metabolites microbiologically active?
  • MIC data from standard test methods

Step 2: Do residues enter the human colon?
  • Absorption, distribution, metabolism, excretion, or bioavailability data
  • If human data is not available appropriate animal data may be used

Step 3: Do residues entering colon remain microbiologically active?
  • In vitro inactivation/metabolism studies of drug incubated with feces
  • In vivo studies evaluating drug’s microbiological activity in feces or colon contents

Step 4: Is there scientific justification to not test endpoints?
  • Barrier disruption and resistance emergence data
  • Test both end-points if cannot eliminate one or the other

Step 5: Calculate microbiological ADI?
  • Determine no-observed-adverse effect concentration (NOAEC) to use in calculation
  • The lowest microbiological ADI value is the value accepted to provide highest safety to human health