Chemical Carcinogenesis

Proportion of chemicals evaluated as carcinogenic

<table>
<thead>
<tr>
<th>Chemicals tested in both rats and mice</th>
<th>Proportion</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naturally occurring chemicals</td>
<td>79/139</td>
<td>57%</td>
</tr>
<tr>
<td>Synthetic chemicals</td>
<td>271/451</td>
<td>60%</td>
</tr>
<tr>
<td>Chemicals tested in rats and/or mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chem. in Carcinogen. Potency Database</td>
<td>702/1348</td>
<td>52%</td>
</tr>
<tr>
<td>Natural pesticides</td>
<td>37/71</td>
<td>52%</td>
</tr>
<tr>
<td>Mold toxins</td>
<td>14/23</td>
<td>61%</td>
</tr>
<tr>
<td>Chemicals in roasted coffee</td>
<td>21/30</td>
<td>70%</td>
</tr>
<tr>
<td>Innes negative chemicals retested</td>
<td>17/34</td>
<td>50%</td>
</tr>
<tr>
<td>Physician’s desk reference PDR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drugs with reported cancer tests</td>
<td>117/241</td>
<td>49%</td>
</tr>
<tr>
<td>FDA database of drug submissions</td>
<td>125/282</td>
<td>44%</td>
</tr>
</tbody>
</table>

Annes and Gold Mutat Rev 447:3-13, 2000

CANCER:

“A multicausal, multistage group of diseases the mechanisms of which are still only partially known” (IARC Scientific Publications, 1992)

“Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells [...] that can result in death” (American Cancer Society, 2002)


- **Benign** tissue is not cancer. Although the cell growth is moderately increased, the cells do not invade nearby tissue or spread to other parts of the body

- **Malignant** tissue is cancer. The cancer cells divide out of control. They can invade and destroy nearby healthy tissue. Also, cancer cells can break away from the tumor they form and enter the bloodstream and lymphatic system

- **Metastasis**: the spread of cancer beyond the organ of origin
**WHAT MAY CAUSE CANCER?**

- Hereditary disorders
- Chemicals
- Viruses
- Chronic inflammation
- ???


**History of Chemical Carcinogenesis**

- Chemical carcinogenesis was first suggested by clinicians 200 years ago
  - Scrotal cancer in chimney sweeps - Potts
  - Nasal cancer and snuff dipping - Hill
  - Today, >50 chemicals are recognized as human carcinogens

- First experimental studies in animals were done ~80 years ago

**History of Chemical Carcinogenesis**

- Large numbers of chemicals were tested for carcinogenic potential in the 1970-1990s
  - Maximum Tolerated Doses (MTD) were used.
  - 60% of rodent carcinogens were genotoxic
  - 40% of rodent carcinogens were nongenotoxic
  - Some chemicals were single site, single species carcinogens
  - Others were multisite, multispecies carcinogens
  - Dose-response varies from <1/2 MTD to <1/1000 MTD

- Most regulations use straight mathematical extrapolation of high dose rodent data to predict risks
IARC (2004)
- Carcinogenic to humans (group 1)
- Probably carcinogenic to humans (group 2A)
- Possibly carcinogenic to humans (group 2B)
- Not classifiable as to its carcinogenicity to humans (group 3)
- Probably not carcinogenic to humans (group 4)

- Carcinogenic to humans
- Likely to be carcinogenic to humans
- Suggestive evidence of carcinogenic potential
- Inadequate information to assess carcinogenic potential
- Not likely to be carcinogenic to humans

- Known to be a human carcinogen
- Reasonably anticipated to be a human carcinogen

- Known to the state to cause cancer

Cancer Cases Attributable to Environmental Carcinogens (Worldwide, 1990)

- Infections (viruses, parasites, H. pylori) 16%
- Tobacco (smoked and smokeless) 14%
- Occupation 4%
- Alcohol drinking 3%
- Diet and dietary components including contaminants 25%
- Pollution 2%
- Reproductive factors 2%

Total: 37%

IARC Group 1 – Carcinogenic to humans
Monographs Volumes 1-84 (1972-2002): 89 Agents and Exposures

- Medical drugs and treatments 24
- Industrial processes 13
- Infectious agents or processes 10
- Physical agents 10
- Industrial chemicals 7
- Inhaled particulates 5
- Metals and inorganic salts 5
- Lifestyle factors (incl. herbal remedies) 7
- Other 8

Note: Group 1: carcinogenic to humans
Group 2A: probably carcinogenic to humans
Group 2B: possibly carcinogenic to humans
Group 3: cannot be classified as to its carcinogenicity to humans

*Other relevant data taken into consideration
**Exposures to Chemicals in the Workplace**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Industries and Trades with Proved Excess Cancers and Exposure</th>
<th>Primary Affected Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Aminodiphenyl</td>
<td>Chemical manufacturing</td>
<td>Urinary bladder</td>
</tr>
<tr>
<td>Asbestos</td>
<td>Construction, asbestos mining and milling, production of friction products and cement</td>
<td>Pleura, peritoneum, bronchus</td>
</tr>
<tr>
<td>Arene</td>
<td>Copper mining and smelting</td>
<td>Skin, bronchus, liver</td>
</tr>
<tr>
<td>Alkylating agents (methylchloroethamine hydrochloride and bis[chloromethyl]ether)</td>
<td>Chemical manufacturing</td>
<td>Bronchus</td>
</tr>
<tr>
<td>Benzene</td>
<td>Chemical and rubber manufacturing, petroleum refining</td>
<td>Bone marrow</td>
</tr>
<tr>
<td>Benzidine, β-naphthylamine, and derived dyes</td>
<td>Dye and textile production</td>
<td>Urinary bladder</td>
</tr>
<tr>
<td>Chromium and chromates</td>
<td>Tanning, pigment making</td>
<td>Nasal sinus, bronchus</td>
</tr>
<tr>
<td>Isopropyl alcohol manufacture</td>
<td>Chemical manufacturing</td>
<td>Cancer of paranasal sinuses</td>
</tr>
<tr>
<td>Nickel</td>
<td>Nickel refining</td>
<td>Urinary bladder</td>
</tr>
<tr>
<td>Polynuclear aromatic hydrocarbons from coke, coal tar, shale, mineral oils, and creosote</td>
<td>Steel making, roofing, chimney cleaning</td>
<td>Skin, scrotum, bronchus</td>
</tr>
<tr>
<td>Vinyl chloride monomer</td>
<td>Chemical manufacturing</td>
<td>Liver</td>
</tr>
<tr>
<td>Wood dust</td>
<td>Cabinetmaking, carpentry</td>
<td>Nasal sinus</td>
</tr>
</tbody>
</table>

Modified from Cullen et al. (1990).

**Carcinogenic Risks of Chemical Agents Associated with Medical Therapy and Diagnosis**

<table>
<thead>
<tr>
<th>Chemical or Drug</th>
<th>Associated Neoplasm</th>
<th>Evidence for Carcinogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkylating agents (cyclophosphamide, melphalan)</td>
<td>Bladder, leukemia</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Boric acid arsanals</td>
<td>Skin, liver</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Azathioprine (an immunosuppressive drug)</td>
<td>Lymphoma, retinoblastoma, skin, Kapost’s sarcoma (?)</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>Bladder</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Leukemia</td>
<td>Limited</td>
</tr>
<tr>
<td>Diethylstibesterol</td>
<td>Vagina (clear cell carcinoma)</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Estrogen</td>
<td>Liver cell adenoma</td>
<td>Limited</td>
</tr>
<tr>
<td>Methoxypridolanes with ultraviolet light</td>
<td>Skin</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Oxamidephosphate</td>
<td>Liver</td>
<td>Limited</td>
</tr>
<tr>
<td>Phenytoin (diphenyhydantoin)</td>
<td>Lymphoma, neuroblastoma</td>
<td>Limited</td>
</tr>
<tr>
<td>Thorotras</td>
<td>Liver (angiosarcoma)</td>
<td>Sufficient</td>
</tr>
</tbody>
</table>

Modified from Pitot (1986a) and Vainio et al. (1991).

**Carcinogenic Factors Associated with Lifestyle**

<table>
<thead>
<tr>
<th>Chemical, Physiological Condition or Natural Process</th>
<th>Associated Neoplasm</th>
<th>Evidence for Carcinogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholic beverages</td>
<td>Esophagus, liver, oropharynx, larynx</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Aflatoxins</td>
<td>Liver</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Betel chewing</td>
<td>Mouth</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Dietary intake (fat, protein, calories)</td>
<td>Breast, colon, endometrium, gallbladder</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Reproductive history</td>
<td>Breast, ovary</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Late age at first pregnancy</td>
<td>Mouth, pharynx, larynx, esophagus, bladder</td>
<td>Sufficient</td>
</tr>
</tbody>
</table>

Modified from Pitot (1986a) and Vainio et al. (1991).

**Chemical Carcinogenesis in the 21st Century**

New perceptions of previously known carcinogens: Combined effects of multiple exposures

Examples:
- Alcohol drinking and aflatoxins
- Alcohol drinking and HBV/HBC
- Alcohol drinking and tobacco smoking
- Tobacco smoking and asbestos/arsenic/radon
Stages of Carcinogenesis

Initiating Event

Cell Proliferation (clonal expansion)

Second Mutating Event

"N" Mutating Event

Promotion

Progression

Malignancy

Cellular and Molecular Mechanisms in Multistage Carcinogenesis: INITIATION

Initiating event involves cellular genome – MUTATIONS

Target genes:
- oncogenes/tumor suppressor genes
- signal transduction
- cell cycle/apoptosis regulators

“Simple” genetic changes

SOURCES OF MUTATIONS

ENDOGENOUS DNA DAMAGE

EXOGENOUS DNA DAMAGE

Free Radicals

Polymerase Errors

Depurination

Environmental Agents

Life Styles

DNA REPAIR

CELL REPLICATION

MUTATION

Chemical Exposure (air, water, food, etc.)

Internal Exposure

Metabolic Activation

DNA

RNA

Protein (Biomarker)

Biologically Effective Dose

Efficiency of Mispairing

Cell Proliferation

Initiation
**Cellular and Molecular Mechanisms in Multistage Carcinogenesis: PROMOTION**

Reversible enhancement/repression of gene expression:
- increased cell proliferation
- inhibition of apoptosis

No direct structural alteration in DNA by agent or its metabolites

```
X = Application of Initiator     Y = Application of Promoter
```

**Accumulation of mutations during tumor progression**

1. X ____________________________ No Tumors
2. X .................................. Tumors
3. X .................................. Tumors
4. .................................... X ____________________________ No Tumors
5. .................................... No Tumors
Cellular and Molecular Mechanisms in Multistage Carcinogenesis: PROGRESSION

- Irreversible enhancement/repression of gene expression
- Complex genetic alterations (chromosomal translocations, deletions, gene amplifications, recombinations, etc.)
- Selection of neoplastic cells for optimal growth genotype/phenotype in response to the cellular environment

Phenotypic characteristics of cancer cells:

- Immortalization
- Transformation
- Loss of contact growth inhibition
- Autonomy of proliferation
- Avoidance of apoptosis
- Aberrant differentiation
- Induction of angiogenesis

Human Tumors and Stages of Carcinogenesis

- Defects in Terminal Differentiation
- Defects in Growth Control
- Resistance to Cytotoxicity

- Activation of Proto-Oncogenes
- Inactivation of Tumor Suppressor Genes
- Inactivation of Anti-metastasis Genes
Multiple Stages of Human Colon Cancer

- It is estimated that by age 70, 50% of the population at large have acquired pre-cancerous adenomas in the colon; 10% of this group will progress to malignancy in the following 10 years.

- Familial Adenomatous Polyposis (FAP) is linked to the APC gene whose protein is involved in β-catenin signaling. The gene acts as a tumor suppressor, and the loss of function mutation causes development of hundreds to thousands of adenomas, with a consequent high risk of progression to malignancy.

- Hereditary Non-Polyposis Colon Carcinoma (HNPCC) is a hereditary predisposition to carcinoma without the prior accumulation of adenoma. HNPCC is caused by a germ line mutation in one set of genes responsible for DNA mismatch repair. To date, there are five genes known to be responsible for causing HNPCC: MSH2, MSH6, MLH1, PMS1 and PMS2. To date, 90% of the inherited mutations in HNPCC are in MSH2 or MLH1.

- Sporadic colorectal cancer (i.e., cancer that occurs without any familial predisposition to the disease) is associated with a variety of risk factors. The most prevalent risk factors, besides a personal or family history of colorectal and specific other cancers, are inflammatory bowel disease and age. Most sporadic colorectal cancers occur in women and men over the age of 50. Additional risk factors include diet, less than moderate exercise, and obesity.

APC protein (Adenomatous Polyposis Coli) is normally expressed in colorectal epithelial cells, a site of relatively high natural proliferation rates. The epithelium is convoluted into deep recesses called crypts and projections called villi. Crypts contain stem cells for tissue replacement, and the base of the crypt is a site of high mitotic activity. As cells age, they progress up the villus to the tip.

Genetic changes underlying development and progression of prostate cancer

- Normal epithelium
- Prostate intraepithelial neoplasia
- Localized adenocarcinoma
- Locally advanced cancer
- Metastatic cancer
- Immunoreactive cancer

By...

- AR
- Androgen and androgenic
- Mutations and androgen...
Histopathological and molecular events leading to esophageal adenocarcinogenesis

Classification of Carcinogens According to the Mode of Action

Mechanism of Carcinogenesis:
Genotoxic Carcinogens

- DNA-reactive or DNA-reactive metabolites
- Direct interaction to alter chromosomal number/integrity
- May be mutagenic or cytotoxic
- Usually cause mutations in simple systems

Classification of Carcinogens According to the Mode of Action

GENOTOXIC:
- DNA-reactive or DNA-reactive metabolites
- Direct interaction to alter chromosomal number/integrity
- May be mutagenic or cytotoxic
- Usually cause mutations in simple systems

Initiation
- Promotion
- Progression
- Malignancy

Mechanism of Carcinogenesis:
Genotoxic Carcinogens

1. Carcinogen activation
2. DNA binding
3. Cell proliferation (fix mutation)
4. Gene mutation

DNA Adduct → Mutation → Cancer
**Interaction of the exo-epoxide of aflatoxin B₁ with DNA**

**Non-Genotoxic Carcinogens**

1) Mitogens:
   - stimulation of proliferation
   - mutations may occur secondarily to cell proliferation
   - may cause preferential growth of preneoplastic cells

2) Cytotoxicants:
   - cytolethal
   - induce regenerative growth
   - mutations may occur secondarily to cell proliferation

**Classification of Carcinogens According to the Mode of Action**

**NON-GENOTOXIC:**
- Do not directly cause DNA mutation
- Mechanism of action is not completely understood
- Difficult to detect - requires rodent carcinogen bioassay

![Mutation Cancer Diagram]

**Tissue Changes with Mitogenic and Cytotoxic Agents**

- Proliferation
- Mitogenic Agent
- Cytotoxic Agent
Mechanism of Carcinogenesis: Non-Genotoxic Carcinogens

Cell proliferation (to fix “spontaneous” mutation) → CANCER

Mechanisms of Non-Genotoxic Carcinogenesis
(what’s in a “black box” ?)
- Increased cell proliferation
- Decreased apoptosis
- Changes in gene expression
- Induction of metabolizing enzymes
- Activation of receptors (signaling)
- Oxidative stress
- ???

Cell Replication is Essential for Multistage Carcinogenesis

- Decreases time available for DNA repair
- Converts repairable DNA damage into non-repairable mutations
- Necessary for chromosomal aberrations, insertions, deletions and gene amplification
- Clonally expands existing cell populations

Mutagenesis ≠ Carcinogenesis
Cell Proliferation ≠ Carcinogenesis
Toxicity ≠ Cell Proliferation
Apoptosis

Programmed Cell Death (Apoptosis): Active, orderly and cell-type-specific death distinguishable from necrotic cell death (passive process):
- Induced in normal and cancer cells
- Non-random event
- Result of activation of a cascade of biochemical, gene expression and morphological events
- tissue and cell specific
- Growth factors and mitogens inhibit apoptosis

Alteration of Gene Expression

- Nuclear (hormone-like) receptors
- Kinase cascades
- Calcium-, nitric oxide-mediated signaling
- Transcription factors
- Gene methylation status (hypo -> enhanced gene expression; hyper -> gene silencing)

Induction of Metabolizing Enzymes

- May be a reason for tissue-, and/or species-selectivity of carcinogens
- Metabolites may be ligands for receptors
- Production of reactive oxygen species

Oxidative Stress

- Indirect DNA damage
- Induction of cell proliferation/apoptosis signaling cascades
The National Toxicology Program (NTP) was established in 1978 to coordinate toxicological testing programs within the Department of Health and Human Services, develop and validate improved testing methods, develop approaches and generate data to strengthen scientific knowledge about potentially hazardous substances and communicate with stakeholders.

- Modified the rodent cancer bioassay
  - More doses
  - Incorporation of pharmacokinetics
  - Incorporation of mechanistic studies
  - Standardization of pathology evaluation
  - More emphasis on non-cancer effects

- Re-evaluate existing practices & research portfolio
  - "Doull" report - 1984
  - Mouse strain workshop - 1985
  - Mechanism conference - 1995
  - NTP Roadmap - August 2003

14-Day Toxicity Protocol

The goal of this is to provide a basis for identifying potential target organs and toxicities and to assist in setting doses for the 13-week exposure study.

**Treatment:**
10- to 14-day quarantine period, animals are assigned at random to groups. Five treatment groups each administered a different concentration of test article per sex/species plus a control group. For dosed-feed and dosed-water studies animals are exposed for 14 consecutive days. For inhalation, gavage and dermal studies animals are exposed for 12 treatment days, not including weekends or holidays with at least two consecutive treatment days before the terminal sacrifice day.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Species</th>
<th>Sexes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Controls</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Observations:**
Animals are weighed individually on day one, after seven days, and at sacrifice. The animals are observed twice daily, at least six hours apart (before 10:00 AM and after 2:00 PM) including holidays and weekends, for morbidity and death. Animals found moribund or showing clinical signs of pain or distress are humanely euthanized. Observations are made twice daily for clinical signs of pharmacologic and toxicologic effects of the chemical. For dosed-feed or dosed-water studies, food consumption/water consumption shall be measured and recorded weekly.

**Necropsy and Histopathologic Evaluation:**
Liver, thymus, right kidney, right testicle, heart, and lung weights are recorded for all animals surviving until the end of the study. A complete necropsy is performed on all treated and control animals that either die or are sacrificed and all tissues are saved in formalin. Histopathologic evaluation is done only on those organs/tissues showing gross evidence of treatment-related lesions to a no-effect level plus corresponding tissues are evaluated in control animals. If specific targets are required they shall be read in the control and highest treatment group and the remaining groups to a no-effect level.

**Early History of Animal Cancer Studies**
- Yamagawa & Ichikawa - 1918
  - Coal tar & SCC of rabbit ears
- Murphy & Sturm - 1925
  - Coal tar skin exposure caused lung tumors in mice
- Cook et al. - 1932
  - PAHs caused skin cancer in mice
- Sasaki & Yoshida - 1935
  - o-Aminodiazotoluene caused liver tumors in rats

**NCI Bioassay History**
- 1952 - First contracted bioassay
- 1969 - Innes et al., study published
  - Selection of B6C3F1 mouse
- 1971 - National Cancer Act
  - Decision made to standardize bioassay testing
- - 1975 - F344 rat selected
  - Small size, vigor & survival, disease resistance
  - Inbred

"The NTP performs appropriate toxicity studies in part to provide dose-setting information for chronic studies and also to address specific deficiencies in the toxicology database for the chemical."

Toxicology/Carcinogenicity studies generally fall into two categories:

1. **Prechronic Toxicity Studies**
   - 14-day study
   - 13 week (90 day) study

2. **Two-Year Toxicology and Carcinogenesis Rodent Studies**
   - usually - ~104 wks
   - sometimes - ~90 wks exposure followed by 10-15 wks of normal diet

**Current NTP Animal Models**
- F344/N@Tac
  - Inbred rat
- B6C3F1/N@Tac
  - Isogenic hybrid mouse
- F1 generation of C57BL/6-J E84 female X C3H/HeN-MTV <- male
In addition to obtaining toxicological data, the purpose of this study is to determine the treatments for each strain and species to be used in the 2-year toxicology/carcinogenesis study.

**Treatment:**
10- to 14-day quarantine period. Animals are assigned at random to treatment groups. Five treatment groups plus a control group. Each group - 10 animals per sex/species. Controls receive untreated water or feed or vehicle alone in gavage and dermal studies. For dosed-feed and dosed-water studies, animals are exposed for 90 days after which they are sacrificed with no recovery period. For inhalation, gavage and dermal studies animals are exposed five times per week, weekdays only until the day prior to necropsy.

**Observations:**
Animals are weighed individually on day 1, after 7 days, and at weekly periods thereafter. Animals are observed twice daily, at least 6 hours apart, including holidays and weekends, for morbidity and death. Formal clinical observations are performed and recorded weekly. For dosed-feed or dosed-water studies, food/water consumption is measured and recorded weekly.

**Necropsy and Histopathologic Evaluation:**
Liver, thymus, right kidney, right testis, heart, and lung weights are recorded from all animals surviving until the end of the study. A complete necropsy is performed on all treated and control animals that die or are sacrificed.

**Specific Toxicological Parameters Evaluated in the 13-Week Study**
Clinical Laboratory Studies: Blood is collected from both sexes of "special study" rats, at days 4 ± 1 and 21 ± 2 and from the core study rats at the end of the study.
Blood for Micronuclei: Blood samples are taken at study termination for micronuclei determinations.
Sperm Morphology and Vaginal Cytology Evaluations (SMVCE)

---

**Two-year Carcinogenesis “Bioassay” Protocol**

- **Typical NTP Bioassay Design**
  - Animal numbers: 50 to 100 per dose group
  - Number of doses: 3 plus control
  - Study duration: 2 years
  - Life stage: young to late adult
  - Dose ranges: MTD, 1/2 to 1/3 or 1/3 to 1/9 MTD
  - Pathology: “complete” approximately 40 tissues
  - Statistics: survival adjusted trend tests
  - Route: feed, gavage, drinking water, inhalation, dermal
  - Diet: NIH-07, NTP-2000
  - Species, strains: F344/N rat, B6C3F1 mouse

<table>
<thead>
<tr>
<th>Test</th>
<th>Animals</th>
<th>Sexes</th>
<th>Species</th>
<th>Groups</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>50 x 2 x 2 x 3</td>
<td>=</td>
<td>600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>50 x 2 x 2 x 1</td>
<td>=</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sentinel Animals</td>
<td>15 x 2 x 1 x 2</td>
<td>=</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>860</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>