Retrospective on Structure Activity Relationships with a Focus on Tamoxifen in the Treatment of Breast Cancer

by

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University of Pittsburgh, 2023

Abstract

Breast cancer is the most common cancer in the United States and worldwide. In women, it is the second leading cause of cancer deaths. Tamoxifen, a selective estrogen receptor modulator, has traditionally been used in the treatment and prevention of breast cancer.

Tamoxifen's triphenylethylene structure activity relationship has been studied to evaluate the correlation between its chemical structure and biological activity. This essay focuses on tamoxifen for the treatment of breast cancer, analyzing its structure activity relationships, associated potential risks (ie. carcinogenicity and mutagenicity) and alternatives.

Through computer-aided structure activity animal models in 1995, tamoxifen was predicted as a carcinogen. Databases were used for each endpoint based on structural alerts. Since then, epidemiological studies have supported the endometrial carcinogenicity of tamoxifen.

Structure activity relationship analyses have proved useful in the risk assessment of tamoxifen. And structure activity relationship models have evolved along with technology. However, there is still room for improvement in decreasing risk to those undergoing breast cancer treatment and preventing new cases. In the discussion, recommendations are made to oncologists on tamoxifen risk, breast cancer treatment options and prevention.

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1.0 Introduction

Computer-based modeling methods relating chemical structure to biological activity have been the foundation for evaluating the risks of environmental hazards, medications, and for safer drug development. Tamoxifen is a selective estrogen receptor modulator drug approved in 1978 for the treatment of metastatic estrogen receptor-positive breast cancer.[1,2] Tamoxifen acts as an antiestrogen in breast cells, attaching to estrogen receptors. This prevents cancer cells from the estrogen needed to grow.[1,3] Tamoxifen was suggested as an endometrial carcinogen in 1985.[4] This risk is the basis of the research conducted and described within this text. There were more than 2.26 million new cases of breast cancer in women worldwide in 2020.[5] In the US from 2013-2020, over 15 million prescriptions of tamoxifen were written to over 3 million patients.[6] Carcinogenicity is an important toxicity endpoint in assessing chemical risk and hazards.[7]

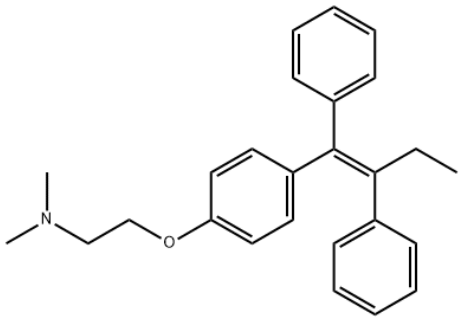

Figure 1. Tamoxifen

(Source: https://www.chemicalbook.com/ChemicalProductProperty_EN_CB9438781.htm)

To address the carcinogenic risks of taking tamoxifen long term, its triphenylethylene structure (Figure 1) was evaluated in the Computer Automated Structure Evaluation and the

MultiCASE software programs. These programs evaluate fragments of the molecule using databases for specific biological endpoints. Biophores, or fragments whose presence may be related to the capability of a substance to cause certain adverse effects to organs, were studied. Structural analyses require these databases to apply known measurements of known molecules. Rodent databases indicated cancer causing potential.

A historical comparison has been made between this earlier cutting edge research and current understanding in order to assess the viability of structural activity relationships in risk analysis. Such analyses have potential far reaching applications. For example, the public health significance of the U.S. government testing less than one percent of registered chemicals for safety or toxicity.[8] In this study, both the carcinogenic risks of tamoxifen and the application of structural analysis are reviewed.

There is value in understanding where we've been to improve the future of public health. Identifying a molecule's mechanism of biological activity through structure function (i.e. receptor binding) is a practical utility of in silico research. As part of this unique retrospective, recommendations are made to oncologists prescribing tamoxifen. They have the option to prescribe aromatase inhibitors, genetic testing and better prevention strategies.

2.0 Backgrounds

2.1 Breast Cancer

Breast cancer is the most common type of cancer for women in the United States and worldwide. Both men and women are at risk, though women make up over 99% of cases. Breast cancer is caused by genetic mutation, resulting in uncontrolled cell growth which forms a malignant tumor. These uncontrolled breast cells can spread to the lymph nodes under the arms and metastasize to other parts of the body via the lymph system. This spread determines the stage of the cancer.[5,9-12]

Worldwide in 2020, there were 2.26 million new cases in women, accounting for 25.8% of the total number of new cases for all cancers.[5] The following is reported in the United States for breast cancer in women[9,10-12]:

- 1 in 8 will develop breast cancer in their lifetime
- 62 is the average age at diagnosis
- 30% of new cancer diagnoses are projected for 2022 with a total of 287,850 new cases
- 85% of breast cancers are due to genetic mutations from aging
- 5-10% of cancers are due to inherited genes (i.e. BRCA)
- Those with a BRCA gene have a 70% lifetime risk
- Breast cancer is the 2nd leading cause of cancer death in women
- Breast cancer is the leading cause of cancer death in Hispanic & African American women

Overall, prognosis has improved. The five year survival rate increased 20% between 1960 and 1990[13] and has now reached an average of 90% for women in the USA. Improvement is due mostly to early detection. The five year survival rate is dependent on the stage of cancer, ranging from an average of 99% for localized growth to 86% for growth spreading to lymph nodes and 30% for metastasis.[11,14]

Both early detection and treatment are key to improving breast cancer survival rates. Standard treatment includes surgery, chemotherapy, radiation and hormone therapy. Treatment differs on many factors, including if the cancer growth is fueled by estrogen or progesterone. Estrogen receptor positive breast cancer relies on estrogen to grow. Therefore, hormone therapy to block the binding of estrogen is often indicated. An estimated 70-80% of breast cancers are hormone receptor-positive.[10-12]

2.2 Tamoxifen

Tamoxifen is a drug approved in 1978 for the treatment of metastatic estrogen receptor-positive breast cancer.[1,2,15,16] Estrogen receptors are different in different parts of the body. For example, estrogen receptors of the breast are different from those in the uterus. Tamoxifen is a selective estrogen receptor modulator (SERM). These medications bind to estrogen receptors and either act like estrogen or block estrogen. Tamoxifen blocks estrogen promoting growth in breast cancer cells by blocking cancer cells from the estrogen needed to grow, therefore acting as an antiestrogen.[1,3,17,18]

As such, tamoxifen is used to treat all stages of hormone receptor-positive breast cancer in women. Tamoxifen hormone therapy has been used in conjunction with surgery and chemotherapy

since the 1980s. Post-surgery, tamoxifen is indicated in the early stage to reduce recurrence. It is also indicated post-surgery in women with ductal carcinoma in situ to reduce the risk of invasive breast cancer.[1,16,19-21]

Early research and clinical trials supported the effectiveness of preventative tamoxifen therapy.[22,23] And in 1998, tamoxifen was the first medication approved by the FDA for the prevention of cancer. Women with a high risk for the disease are eligible for this chemoprevention. Criteria may include a Gail model risk score greater than 1.7%.[15,16,18,24]

Tamoxifen remains on the most recent list of essential medicines by the World Health Organization.[25] It's benefits for breast cancer include[26,27]:

- 40-50% reduced recurrence in post-menopausal women
- 30-50% reduced recurrence in pre-menopausal women
- 50% reduced risk of new cancer in the other breast
- 40% reduced initial diagnosis
- 50% reduced invasive breast cancer for those with noninvasive ductal carcinoma in situ

These benefits may extend up to twenty years following the end of tamoxifen therapy.[28] There is a secondary benefit of tamoxifen for post-menopausal women. In the bones, tamoxifen acts as an estrogen agonist (acting like estrogen) preventing bone loss.[29]

This agonist activity is also seen in the uterus. This estrogen stimulation increases the risk of uterine sarcoma and endometrial cancer (the lining of the uterus). This risk was first noted by Killackey et al. in 1985.[4] And by the 1990s there were many documented reports of endometrial cancer in tamoxifen-treated athymic mice[30] and in women.[4,31] Tamoxifen associated cancer of the uterus is a risk. Its usage has been extended from five years, to up to ten years.[32] On one hand, this

extended therapy has shown a reduction in recurrence and mortality.[33] On the other hand, the risk of endometrial cancer is also extended to an estimated two to seven fold increase.[34]

Tamoxifen is prescribed to patients by oncologists who must weigh the benefits and risks for their patients. In 2020, there were nearly a million prescriptions of tamoxifen in the United States.[6] To the benefit of oncologists, tamoxifen's epidemiology, clinical research, molecular structure and biological activity have all been well documented. And its triphenylethylene structure continues to be evaluated for new drug discovery and repurposing.[35]

2.3 Structure Activity Relationships

A structure activity relationship (SAR) is the connection between a chemical's molecular structure and its biological activity. Scientists have been making these predictions dating back to the 1800s.[36] The analysis is based on the presence or absence of a chemical structure and the presence or absence of a biological activity.

A part of the chemical structure related to a specific biological activity is extracted. This fragment is called a biophore or toxicophore or structural alert. This distinct part of the chemical is theoretically responsible for the biological activity. For example, if chemical "A" has a biophore of known hazard that causes liver toxicity and chemical "B" has the same biophore, then chemical "B" may also cause liver toxicity. Basically, similar compounds may have similar physical and biological endpoints, or outcomes. The absence of the biophore does not mean that a molecule is nontoxic because the whole molecule is not considered.[37]

This qualitative approach characterizes structures as active or inactive. This information and contributing data can be weighted and quantified to predict the potency of the biological

activity. The science of quantitative structure activity relationships, or QSAR, was pioneered 60 years ago by Corwin Hansch.[38] Today, both qualitative and quantitative analyses are used together (and will be referred to under the umbrella of SAR herein). In general, structure activity relationship analyses are conducted in silico, or via computer.

The SAR methodology has many applications in public health. Chemicals are a part of 21st century living. There are over 20,000 drugs and over 350,000 registered chemicals.[39,40] Are they safe? What are the exposure risks? How do we develop safer options? SAR helps to answer those questions by predicting and characterizing chemical toxicity. These risk assessments are used in a wide range of industries, including environmental health and pharmaceuticals. They are fast and low cost compared to the traditional benchmarks of in vitro laboratory testing and in vivo animal experiments. SAR can be used whenever information is needed for a toxicological endpoint (ie. cancer).[41]

3.0 Structural Relationship Components

To assess a chemical such as tamoxifen for a biological endpoint, one needs to relate its chemical structure to that endpoint.

3.1 Software

In the 1980s, Dr. Giles Klopman pioneered artificial intelligence for chemical toxicity predictions. This led to the Computer Automated Structure Evaluation (CASE) and MultiCASE software programs. Each revolutionized toxicology by predicting the biological activity of molecules based on structural fragments. It correlated biological data to organic molecules.[42-45]

The software deconstructs a molecule into every possible fragment from two to ten atoms long (excluding hydrogens). Biophores (also known as alerts) are identified from a learning set database of known structures with known activities. The biophores are chemical fragments contributing to toxicity based on the learning set. CASE identifies any biophore that contributes to activity (with less than a 15% chance of being a random event). In contrast, MultiCASE only uses the most significant biophore in its analysis. The activity of the biophore is not absolute. It can be modulated by fragments and physical chemical properties (i.e. water solubility).[46,47]

For each biophore, the software calculates a predicted level of activity based on what is known. This is specific to one chemical and one endpoint. The result is reported as 1) activity level and 2) percent probability of being active. To do this, one must have a learning set database for

that endpoint with reference chemicals. For example, to know if tamoxifen is an active carcinogen in rodents, one must have databases of in vivo rodent carcinogenicity tests.

3.2 Databases

3.2.1 Carcinogenic Potency Project

While Dr. Klopman revolutionized SAR, there were other pioneers contributing to SAR research. These include biochemist Bruce Ames and Lois Swirsky Gold. In the 1970s Bruce Ames developed the *Salmonella* mutagenicity assay, or Ames test.[48] A few years later, Bruce Ames initiated the Carcinogenic Potency Project which was shortly thereafter run by Lois Gold.[49] This project was the first of its kind. It was a database of all chemical toxicity studies conducted on animals worldwide. The project was a compilation of animal cancer tests, including 770 test compounds in 3,000 long-term experiments. The project was first published in 1984 by twelve authors, including Bruce Ames.[50] The project database is herein referred to as "Gold." Lois became a worldwide expert on rodent carcinogens and the number of experiments grew to 4,000 with 1,050 test compounds.[51]

The Gold databases are based on absolute carcinogenic activity, and therefore of great interest to this investigation. A molecule must show carcinogenicity to rats or mice or both to be considered active. Therefore, the potency of a chemical as a carcinogen can be derived. Each rodent database relates a threshold dose to CASE units. The threshold dose (TD_{50}) induces cancer in half of the test animals by the end of their species' lifespan (Table 1).[52]

Table 1. Derivation of CASE activity units in Gold carcinogenicity databases

Gold Database	CASE Activity Derivation
Rat	CASE Activity units = 20.1236 x Log(1/TD_{50}) + 44.0658
Mouse	CASE Activity units = 14.1329 x Log(1/TD_{50}) + 44.1329
Rodent	CASE Activity units = 18.3279 x Log(1/TD_{50}) + 46.5517

With respect to CASE units, three classifications of activity exist. Active carcinogens range from 30 to 99 CASE units, marginally active molecules range from 20 to 29 CASE units and inactive molecules (non-carcinogens) range from 10 to 19 CASE units.

3.2.2 National Toxicology Program

The second collection of rodent carcinogenicity databases came from the U.S. National Toxicology Program of the National Institute of Environmental Health Sciences. This is herein known as NTP. These databases were founded in 1978 with the purpose of sharing toxicological data for potentially hazardous substances. In 1995 program materials became available online.[53,54] In contrast to the Gold databases, the NTP databases relate a spectrum of biological activity to CASE units (Table 2).

Table 2. CASE activity in the National Toxicology Program rodent carcinogenicity databases

CASE Activity Units	Biological endpoint
60	Chemical carcinogenic to rats and mice at ≥1 site
50	Chemical carcinogenic to rats or mice at ≥2 sites
40	Chemical carcinogenic to rats or mice at 1 site in both sexes
30	Chemical carcinogenic to 1 site in 1sex of 1 species

20	Chemicals with uncertain evidence of carcinogenicity
10	Non-carcinogenic chemicals

3.2.3 *Salmonella* Assay

As mentioned above, the Ames test was developed in the 1970s. The assay uses *Salmonella* bacteria to test if a chemical produces mutations. Mutations cause changes, or damage to a cell's DNA. The test identifies a wide range of chemicals. All chemicals causing mutation to the cell are considered genotoxic.[48,55] This genetic damage can lead to cancer.

3.2.4 DNA Reactivity

Similar to the *Salmonella* assay, the DNA reactivity database identifies genotoxicity in which a chemical structure reacts with DNA. This learning set is a compilation of alerts, or biophores, known to react with DNA as defined by Ashby and Tennant in the 1980s. Each chemical was evaluated within the software for potential DNA reactive sites (i.e. those that are electrophilic) which are suggestive of carcinogenic activity. Ashby and Tennant found a correlation between DNA reactivity and mutagenicity.[56-58] The significance of genotoxicity is the threshold for causing cancer (Figure 2).

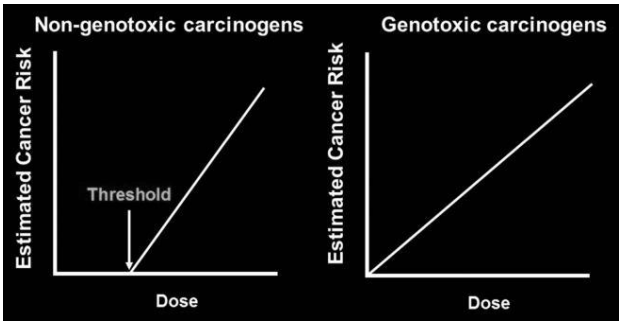

Figure 2. Estimated dose for cancer causation of genotoxic versus non-genotoxic chemicals

(Source: "Models for dose-response curves of non-genotoxic and genotoxic carcinogens." by Nohmi T., https://doi.org/10.5487%2FTR.2018.34.4.281)

Genotoxic compounds do not have a threshold, meaning theoretically only one molecule can cause tumor growth via DNA damage and mutations. Whereas non-genotoxic compounds cause tumor growth by mechanisms requiring a higher dose. Therefore, genotoxic carcinogens pose a high risk to humans.[59]

3.3 Organic Compounds

The primary application of structural analysis relationships are to organic compounds. SAR analyses can be made of one molecule or more at a time, with analogue comparisons being a useful tool. Even slight modifications to a molecule can affect its activity (i.e. estrogen binding). By identifying a molecule's activity, more derivatives of the base compound can be made and toxic derivatives can be avoided. This practical application for pharmaceuticals extends to DNA reactivity, carcinogenicity, binding affinity and more.

Toremifene is a selective estrogen receptor modulator created for improved safety (i.e. genotoxicity and carcinogenicity) over tamoxifen. Its chemical structure only differs from

tamoxifen by the addition of a chlorine atom (Figure 3). Each shares many of the same properties. Toremifene was approved in 1997 for treatment of metastatic breast cancer, making it the first SERM on the market since tamoxifen in 1978.[60] This investigation focused on tamoxifen safety with consideration for toremifene.

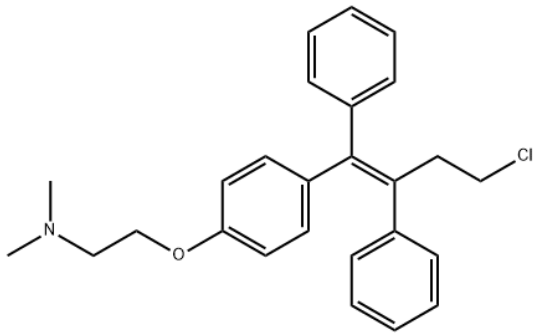

Figure 3. Tormeifene

(Source: https://www.chemicalbook.com/ChemicalProductProperty_EN_CB0287854.htm)

4.0 Structure Activity Analysis

If information about tamoxifen's carcinogenic risks is to reach breast cancer patients, it must first be acknowledged by oncologists prescribing its long-term usage. This two-fold analysis compares the following: 1) historical data in the CASE and MultiCASE systems for tamoxifen and toremifene with a focus on carcinogenicity and 2) a comparison of SAR usage for risk analysis then and now.

4.1 CASE and MultiCASE Analysis

The triphenylethylene tamoxifen was applied to the Computer Automated Structure Evaluation (CASE) and the MultiCASE software. In tandem with this analysis, the chlorinated triphenylethylene toremifene was analyzed by the same parameters. At the time, research sought an effective and less toxic selective estrogen receptor modulator to tamoxifen.

4.1.1 Method

In the parent compound analysis of tamoxifen and toremifene, six databases were used. Each was specific to a biological endpoint. As both genotoxic and mutagenic molecules may lead to cancer, both the database for DNA reactivity and the Ames *Salmonella* assay for mutations were used. Three of the Gold animal carcinogenicity databases were utilized, specifically for rats, mice and rodents. The National Toxicology Program's rodent carcinogenicity database was also used.

The CASE and MultiCASE software identified molecular fragments from tamoxifen and toremifene within each database. The biophores contributing to molecular activity were evaluated. Results were then summarized for each molecule in each database as both CASE activity units and the percent probability of being active.

Further investigation into tamoxifen (and toremifene) carcinogenicity led to a metabolite analysis. The metabolites used for analysis were those suggested by Lim et al[61] and those generated by the META program. The META program was developed by Klopman and associates.[44] It uses the molecular structure of an organic compound to predict metabolites formed from biological transformations. In total, 88 tamoxifen metabolites and 87 toremifene metabolites were imputed into the following four databases: 1) *Salmonella* assay, 2) DNA reactivity, 3) Gold rodent and 4) NTP rodent.

4.1.2 Result

In the parent compound analysis of tamoxifen and toremifene, cancer causing potential was found in all rodent databases. The genotoxic and mutagenic potential was mixed with lower activity levels. The summary of these parent compound results are provided in Table 3.

Table 3. Summary of CASE and MultiCASE results for Tamoxifen and Toemifene in databases for carcinogenicity, mutagenicity and DNA reactivity

Database	Tamoxifen				Toremifene			
	CASE		MultiCASE		CASE		MultiCASE	
DNA Reactivity	36%	10	*		79%	21	87%	39
Salmonella Assay	42%	10	*		42%	10	76%	16
Gold-Rat Carcinogenicity	78%	26	**		92%	42	**	
Gold-Mouse Carcinogenicity	68%	54	*		89%	72	*	
Gold-Rodent Carcinogenicity	98%	61	80%	76	100%	81	**	
NTP-Rodent Carcinogenicity	***		71%	47	***		71%	49

* No known biophore in MultiCASE
** Overruled by CASE
***Overruled by MultiCASE

The results in the DNA reactivity database were very different for tamoxifen and toremifene. Tamoxifen was predicted to be inactive. In contrast, toremifene had high probability of DNA reactivity in both MultiCASE and CASE, though with lower activity levels.

In the *Salmonella* assay, tamoxifen was not predicted to be mutagenic. Toremifene could not be substantiated as a mutagen due to chemical structure, as the majority of molecules with the reference biophore in the *Salmonella* database were aliphatic and toremifene is an aromatic.

In the Gold rat database, both tamoxifen and toremifene were predicted to be extremely active in MultiCASE, with 164 and 184 CASE units, respectively. However, their shared biophore was questionable in the database and thus gave a flawed result. Standard operating procedure overrules MultiCASE with CASE. As such, CASE predicted a 92% and 78% probability of being a rat carcinogen for toremifene and tamoxifen, respectively.

In the Gold mouse database, tamoxifen and toremifene did not contain any known biophores in MultiCASE. Biophores were present, but disqualified due to conformational

differences. Therefore, both were presumed inactive. And although tamoxifen and toremifene were found to be inactive in MultiCASE, they were active in CASE. Tamoxifen was predicted to be very active with 54 CASE units and a 68% probability of being a mouse carcinogen. Toremifene's probability of being a mouse carcinogen was 89% with 72 CASE units of activity.

In the Gold rodent database a molecule must show carcinogenicity to rats or mice or both to be considered active. High carcinogenic activity was found for both tamoxifen and toremifene. Tamoxifen was an active carcinogen with a 98% and 80% probability of being a rodent carcinogen in CASE and MultiCASE, respectively. Toremifene carcinogenicity could not be substantiated in MultiCase and therefore CASE is the primary result. In CASE, toremifene was predicted to be an extremely active carcinogen with 81 units of activity and a 100% probability of being a rodent carcinogen.

Tamoxifen and toremifene had a 71% probability of being a rodent carcinogen in the NTP database for MultiCASE. CASE was inconclusive and thus overruled. Both were predicted to be very active, with 47 CASE units of activity for tamoxifen and 49 units for toremifene. These activities indicated carcinogenicity to rats or mice at a single site in both sexes.

The META program metabolized tamoxifen along several pathways. These included its two main metabolites known today as 4-hydroxy-tamoxifen and 4-hydroxy-N-desmethyl tamoxifen. The latter is now better known as endoxifen. Each possesses greater binding affinity to the estrogen receptor than tamoxifen itself. Both therefore have more antiestrogenic effects in breast cancer cells.[62] Tamoxifen's primary metabolites are illustrated in Figure 4.

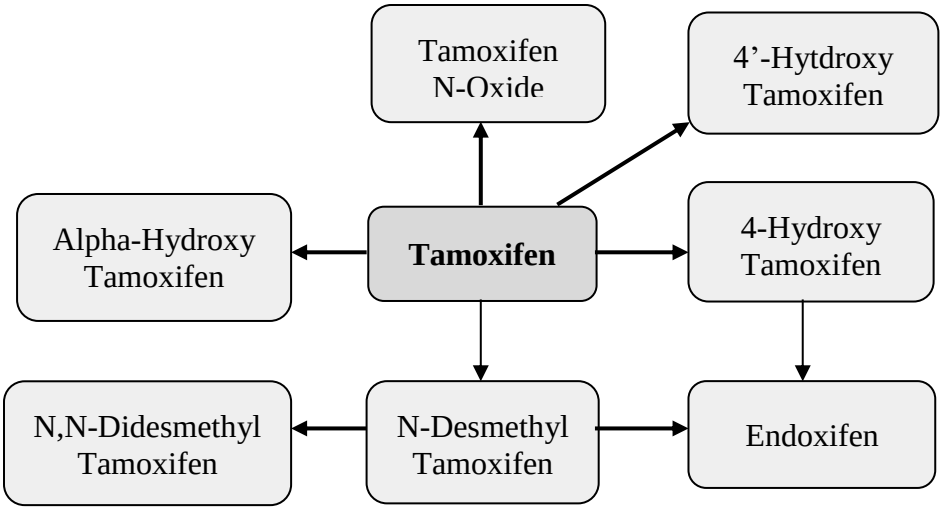

Figure 4. Metabolism tree of Tamoxifen

(Source: https://www.pharmgkb.org/pathway/PA145011119)

Tamoxifen metabolites had a positive response in the *Salmonella* assay for mutagenicity. However, upon further investigation, they were either inconclusive, subject to low confidence levels based on the positivity of too few chemicals, or false positives. This illustrates the limitation of the database at the time. Toremifene metabolites were unsubstantiated as mutagens, as was toremifene.

Secondary metabolites of N-desmethyl tamoxifen were active for genotoxicity in the DNA reactivity database. However, the activity was unsubstantiated due to the low confidence levels of a small sample set. Toremifene metabolites, as was the parent compound, were highly DNA reactive in both MultiCASE and CASE.

The Gold and NTP carcinogenicity data for tamoxifen along its main metabolic pathways is provided in Table 4. CASE activity and percent probability of being active for each database endpoint is given. Tamoxifen (and toremifene) metabolites were active for carcinogenicity.

Table 4. Summary of CASE and MultiCASE results for primary metabolites of tamoxifen in the Gold and NTP rodent carcinogenicity databases

Tamoxifen Metabolite	Gold Rodent Carcinogenicity					NTP Rodent Carcinogenicity			
	CASE		MultiCASE			CASE		MultiCASE	
4-Hydroxy Tamoxifen	99%	84	80%	64		*	*	86%	45
N-Desmethyl Tamoxifen	97%	61	80%	60		35%	22	68%	*
Endoxifen (4-Hydroxy-N-Desmethyl Tamoxifen)	99%	84	80%	78		35%	22	68%	*
N,N-Didesmethyl Tamoxifen	97%	61	80%	60		80%	48	85%	49
Alpha-Hydroxy Tamoxifen	96%	61	80%	45		38%	*	86%	44
4'-Hydroxy Tamoxifen	99%	84	80%	64		80%	48	86%	45
Tamoxifen N-Oxide	94%	61	80%	60		*	*	86%	49

* = Inactive

In the Gold rodent database for carcinogenicity, 88% of tamoxifen metabolites were active in both MultiCASE and CASE. The seven primary metabolites showed high carcinogen probabilities in CASE (94-99%) and MultiCASE (80%). As with the parent compound analysis, toremifene metabolites showed preference to MultiCASE and stronger activity than those of tamoxifen.

Tamoxifen and toremifene metabolites were active in the rodent carcinogenicity database of the National Toxicology Program (NTP). In MultiCASE 60% of the tamoxifen metabolites had an 85% chance or better of being rodent carcinogens. Fifty percent had potencies in the forties, indicating carcinogenicity to rats or mice at two or more sites. This corresponded with the potency of tamoxifen (47 units). Toremifene metabolites mirrored tamoxifen, with 57% having potencies in the forties and thus carcinogenic to rats or mice at two or more sites.

Overall, this study predicted tamoxifen to be a carcinogen in rodents, supporting knowledge from that time. Tamoxifen showed a high probability of carcinogenic activity in the NTP rodent and Gold rat, mouse and rodent databases. This activity was not attributed to one biophore. Metabolism of tamoxifen did not indicate causation. The META analysis showed that the metabolites were easily deactivated and very rarely became more active than the parent compound. The metabolites retained their carcinogenicity when tamoxifen and toremifene were already predicted to be a carcinogen. It was concluded that the metabolites of tamoxifen and toremifene were not responsible for activity in the databases. Therefore, other factors were anticipated to be responsible for the activity.

At the time, toremifene was considered as a possible substitute for tamoxifen in the treatment of breast cancer. However, this study did not support the safety of toremifene any more than that of tamoxifen.

The purpose of this experiment was to decrease the uncertainty in risk assessment and to make sure that humans are not exposed to unnecessary risks. This was a mechanistic problem and using structure activity relationships instead of spending a great deal of money on animal testing was very advantageous. And while one mechanism was not indicated, this study supported the rodent carcinogenicity of tamoxifen and its analogue toremifene. How the animal database results relate to humans was unknown. Therefore, tamoxifen safety in humans was left uncertain, at best. Other factors were anticipated to be responsible for the activity.

4.2 Limitations

It was later discovered that tamoxifen is both an estrogen agonist and antagonist. This made tamoxifen a unique challenge in structural activity relationships. The main limitations of this type of analysis was not accounting for species differences or estrogen receptor binding. In addition, technology was a significant limiting factor. Earlier versions of the CASE and MultiCASE programs had limitations that affected outcomes. For example, results were often flawed due to biophores being labeled as questionable by a program that had no imputed data for its structure. At times, a particular biophore was considered carcinogenic in either the NTP or Gold databases because just one out of the known molecules containing that biophore was carcinogenic in the database. Results in CASE or MultiCASE can be overruled by the other due to substructure modulators, incorrect conformation and conformational differences. Additionally, the size of the biological endpoint databases were significantly smaller than today. Fewer organic compounds with known fragments and fewer animal studies both were limiting.

4.3 Comparison of SAR Then and Now

The software and databases grew over time with the exponential growth of technology. Just as computers grew from a couple hundred megabytes of storage in 1994 to multi-terabyte today,[63] database information grew as well. In 2019, there were nearly 1000 databases for in silico research (Figure 5).

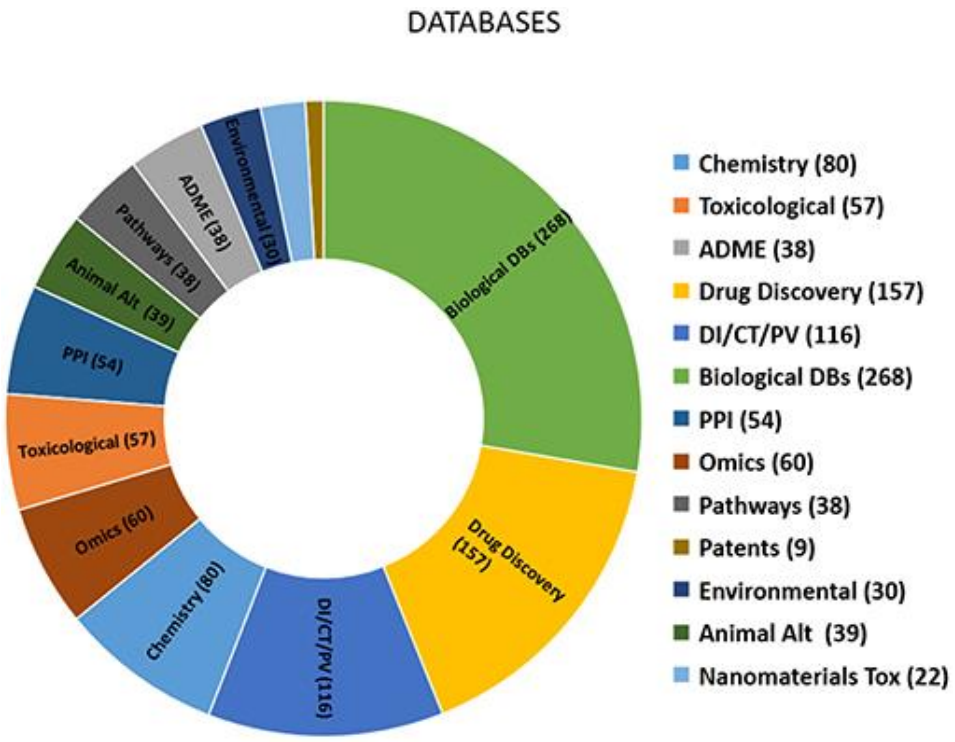

Figure 5. Databases available for in silico chemical and drug safety assessments

(Source: Gopal, P., Madden, J.C., Ebbrell, D., Firman, J.W., and Cronin, M.T.D. "FIGURE 1. Chart showing the number of databases within each group. DI, Drug Information; CT, Clinical trials; PV, Pharmacovigilance; PPI, Protein-protein interactions; Animal Alt, Animal alternatives." In Silico Toxicology Data Resources to Support Read-Across and QSAR. Frontiers in Pharmacology, vol. 10, 2019. https://doi.org/10.3389/fphar.2019.00561)

The Gold rodent carcinogenicity database became known as the Carcinogenic Potency Database. According to Bruce Ames, the database was used by every regulatory agency in the world. Lois Gold became known as the world's expert on the potency of rodent carcinogens.[49] Following her death in 2012, the database was taken over by Lhasa Limited in 2016 and is now a free resource known as the Lhasa Carcinogenicity database with 7,745 long-term carcinogenicity studies for 1,726 chemicals.[64]

The National Toxicology Program still uses long-term rodent studies to identify carcinogenicity and is developing computer-based predictive toxicology models to decrease

animal use. NTP has collaborated since 2004 on the Toxicology in the 21st Century program (Tox21) to develop new rapid testing methods of chemicals.[65] Both Tox 21 and the Lhasa carcinogenicity database are included in the toxicological databases of Figure 5.

The AMES mutagenicity test is still common in many toxicological laboratories around the world with recent assays available for oil testing and mutagenic activity of water extracts.[66]

In 1996, Dr. Gilles Klopman of the META program, consolidated the CASE software and his META program into MultiCASE inc. The MultiCASE platform is still in use and includes CASE Ultra for QSAR analyses and META Ultra for metabolite predictions. The software is used in FDA collaboration and regulatory compliance.[44,67,68]

Structural alerts have been widely applied to drug discovery, toxicity, cosmetic research and environmental protection.[69-72] Chemical structure based modeling is not only standard practice in drug discovery and development, but is part of the approval process for the U.S. Food and Drug Administration.[73] There are now international guidelines set forth by the FDA and the International Council for Harmonization (ICH), *"that allow drug developers to substitute predictions based on quantitative SAR models for traditional laboratory tests when determining the mutagenicity of drug impurities.[74]"* SAR research conducted decades ago laid a foundation that was later advanced with technology.

5.0 Discussion

SAR's application is more important now than ever. Drug discovery is a long and costly process that takes about 14 years. The average cost to develop each new drug is $1–2 billion. The failure rate during development is nearly 96%.[75,76] The inability of animal models to accurately predict human disease contributes to this high failure rate. Standard animal cancer research involves inducing tumor growth into an animal for study. These models are limited in their ability to translate cancer in animals to the complexities of human carcinogenesis. In fact, 90% of drugs fail in humans after animal testing due to species differences.[77-79]

The National Center for Advancing Translational Services at the National Institutes of Health is an institute established in 2011 to expedite drug discovery to get treatments to patients faster.[80] A new methodology is structure–tissue exposure/selectivity relationship (STR). This focuses on drug tissue exposure and selectivity in disease targeted tissues. Understanding how structural properties relate to specific tissue accumulation improves drug efficacy. A proposed combination of SAR and STR would use imaging of human tissue in the future.[81]

For now, animal tests are still required before most chemicals can be used in drugs, cosmetics, etc. But animal testing is being minimized worldwide in favor of sustainable life cycle management. In order to stay on trend, researchers must find alternative methods to evaluate toxic properties of chemicals with specific endpoints such as carcinogenesis. This is evident in the cosmetic industry. Eight states have passed laws banning cosmetics animal testing and 42 countries have banned or limited such testing (including Europe, Australia, Mexico and India).[82]

Scientifically the 96% failure rate in clinical drug testing suggests reevaluating animal modeling, but what of ethics? It is estimated that more than 115 million animals worldwide are

used in laboratory experiments every year. Actual numbers are suspected closer to 1 billion, as the US only reports 10%.[83] Mahatma Gandhi said that “the greatness of a nation and its moral progress can be judged by the way its animals are treated." In silico models using AI and in vitro methods with human tissue are aligned with a more successful, timely, affordable and moral future.

Tamoxifen was developed with SAR modeling and its structure has given rise to a new generation of SERMs (i.e. raloxifene). Tamoxifen’s unique dichotomy of treating and causing cancer via its estrogen pathway calls our acceptance of risk into question. Tamoxifen is a Group 1 known human carcinogen.[84] Although the benefits of tamoxifen are evident and uterine cancer has a significantly better prognosis than breast cancer,[85] the practice of oncologists treating cancer with a known carcinogen is less than ideal. Current hormonal therapy alternatives for oncologists to prescribe are raloxifene, aromatase inhibitors and estrogen receptor downregulators. Oncologists may also prescribe pharmacogenomic testing to predict drug response based on genetics.

Oncologists and the public must decide each day on what is an acceptable risk in the 21st century. While tamoxifen may be a lifesaving drug, Group 1 carcinogens are all around us. They include x-rays, sunlight, tobacco, alcohol and processed meats. Additionally, death from doctor prescribed medications was recently the 4th leading cause of death in the US with approximately 128,000 deaths/year. That’s triple the breast cancer death rate.[86] It is time to ask if the benefits of our choices outweigh the risks. But how many people make the connection between Group 1 carcinogenic processed pepperoni on pizza today and developing cancer years from now?

In terms of breast cancer risk, early detection and treatment have undoubtedly increased survival. However, the big picture of all the statistics is the number of new cases. In twenty years this has not changed beyond a small margin. The number of new breast cancer cases per 100,000 women in the United States has averaged 126.1 with a consistent trend line (Figure 6).[85]

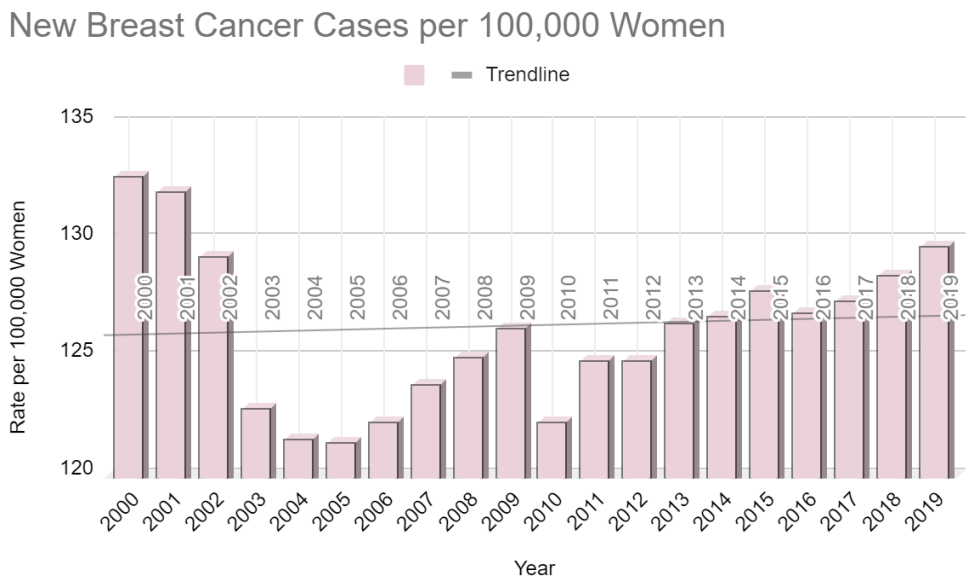

Figure 6. Annual number of age-adjusted new female breast cancer cases in the United States from 2000-2019

(Source: https://gis.cdc.gov/Cancer/USCS/#/Trends)

Therefore, it is time to focus on prevention, which means never getting breast cancer. A new medical strategy is needed. The number one risk factor is being a woman over 50 years of age. Aging is the risk factor. Eighty-five percent of cases are due to age. Few medications are approved for prevention (i.e. tamoxifen, raloxifene, exemestane, and anastrozole) and only for those women with above average risk. Many prevention strategies have been studied. These include avoiding a sedentary lifestyle, alcohol and hormone replacement therapy while getting regular exercise and maintaining a healthy adult weight.

Hundreds of thousands of research studies have also been conducted on aging, cancer mechanisms and supplementation. Those showing promise for breast cancer included, but are not limited to the following:

- 3,3′-Diindolylmethane (DIM) - for healthy estrogen metabolism
- Cruciferous vegetables (i.e. cabbage, kale, broccoli, brussel sprouts)

- Sulforaphane - sulfur compound in cruciferous vegetables
- Soy isoflavones
- Green tea
- High lignan (phytoestrogen) flaxseed oil
- Turmeric
- Advanced glycation end-product prevention (i.e. benfotiamine, carnosine)
- Antioxidants to combat free radical damage
- Molecular Iodine

Despite an aging population, one day there does not need to be 2.26 million new cases of breast cancer in the world. Prevention options exist from hormonal therapy to lifestyle changes to supplementation. It's time to integrate holistic approaches and medicine into a new era of prescriptions before we're 50.

6.0 Conclusion

Oncologists, researchers, public health professionals and policy makers are tasked to redefine 20th century precepts:

1. In silico SAR is a useful approach that has been validated over time. It is part of a future that must take cost, time, success rates and 21st century ethics into consideration.
2. Risks are all around us. Breast cancer needs a new strategy of prevention and integration. If the most common risk of developing breast cancer is simply to be a woman over 50 years of age, a waiting to detect approach will not improve incidence. Prevention strategies are called for. Many are available at low to no cost.

Bibliography

[1] Furr, B.J.A. and Jordan, V.C. “The pharmacology and clinical uses of tamoxifen.” Pharmacology & Therapeutics, vol. 25, no. 2, 1984, pp. 127-205. https://doi.org/10.1016/0163-7258(84)90043-3.

[2] Harper, M.J.K. and Walpole, AL. “Contrasting endocrine activities of cis and trans isomers in a series of substituted triphenylethylenes.” Nature London, vol. 212, no. 87. 1966. https://doi.org/10.1038/212087a0.

[3] Kallio, S., Kangas, L., Blanco, G. et al. “A new triphenylethylene compound, Fc-1157a.” Cancer Chemotherapy and Pharmacology, vol. 17, no. 2, 1986, pp. 103–108. https://doi.org/10.1007/BF00306736.

[4] Killackey M.A., Hakes T.B., Pierce V.K. “Endometrial adenocarcinoma in breast cancer patients receiving antiestrogens.” Cancer Treatment Reports, vol. 69, no. 2, 1985, pp. 237–238.

[5] Cancer trends, World Cancer Research Fund International. https://www.wcrf.org/cancer-trends/.

[6] Tamoxifen-drug usage statistics, United States, 2013 - 2020. ClinCalc.com. https://clincalc.com/DrugStats/Drugs/Tamoxifen.

[7] Plošnik, A., Vračko, M., Sollner Dolenc, M. “Mutagenic and carcinogenic structural alerts and their mechanisms of action.”Arhiv za Higijenu Rada i Toksikologiju, vol. 67, no. 3, 2016, pp. 169-182. https://doi.org/10.1515/aiht-2016-67-2801.

[8] “Blowing smoke: chemical companies say trust us, but environmental and workplace safety violations belie their rhetoric.” Center for effective government. October 22nd, 2015, page 2. https://www.foreffectivegov.org/blowing-smoke.

[9] Breast cancer information and statistics. Centers for Disease Control and Prevention. https://www.cdc.gov/cancer/breast/index.htm.

[10] Breast cancer information, facts and statistics. Breastcancer.org https://www.breastcancer.org/about-breast-cancer.

[11] Breast cancer information and statistics. Susan G. Komen foundation. https://www.komen.org/breast-cancer/.

[12] About breast cancer-cancer basics, types, research and statistics. American Cancer Society. https://www.cancer.org/cancer/breast-cancer/about.html.

[13] Wingo PA, Tong T, Bolden S. Cancer statistics, 1995. CA-A Cancer Journal for Clinicians, vol. 45, no.1, 1995, Jan-Feb, pp. 8-30. https://doi.org/10.3322/canjclin.45.1.8.

[14] Cancer Stat Facts: Female Breast Cancer. National Cancer Institute. Surveillance, Epidemiology and End Results Program. https://seer.cancer.gov/statfacts/html/breast.html.

[15] "Tamoxifen." Britannica, The Editors of Encyclopedia. Encyclopedia Britannica, 10 Oct. 2022. https://www.britannica.com/science/tamoxifen.

[16] Quirke, V. "Tamoxifen from Failed Contraceptive Pill to Best-Selling Breast Cancer Medicine: A Case-Study in Pharmaceutical Innovation." Frontiers in Pharmacology, 0 vol. 8, 2017, pp. 620. https://doi.org/10.3389/fphar.2017.00620.

[17] Ki-Chan An. "Selective Estrogen Receptor Modulators." Asian Spine Journal, vol. 10, no. 4, Aug 2016, pp. 787–791. http://doi.org/10.4184/asj.2016.10.4.787.

[18] Breast Cancer Risk and Prevention. American Cancer Society. https://www.cancer.org/cancer/breast-cancer/risk-and-prevention.html.

[19] Treatment for breast cancer-Hormone Therapy. Cancer Research UK. https://www.cancerresearchuk.org/about-cancer/breast-cancer/treatment/hormone-therapy.

[20] Breast cancer treatment-Tamoxifen. Susan G. Komen foundation. https://www.komen.org/breast-cancer/treatment/type/hormone-therapy/tamoxifen/.

[21] Heel RC, Brogden RN, Speight TM, Avery GS. "Tamoxifen: a review of its pharmacological properties and therapeutic use in the treatment of breast cancer." Drugs, vol. 16, no. 1, 1978, pp. 1-24. http://doi.org/10.2165/00003495-197816010-00001.

[22] Cuzick J, Wang DY, Bulbrook RD. "The prevention of breast cancer." Lancet, vol. 1, no. 8472, 1986, pp. 83-86. http://doi.org/10.1016/s0140-6736(86)90729-4.

[23] Powles, TJ, Tillyer, CR, Jones, AL, Ashley, SE, Treleaven, J, Davey, JB and McKinna, JA. "Prevention of breast cancer with tamoxifen - an update on the Royal Marsden Hospital pilot programme. European Journal of Cancer, vol. 26, no. 6, 1990, pp. 680-4. http://doi.org/10.1016/0277-5379(90)90116-b.

[24] Breast cancer information-Estimating breast cancer risk. Susan G. Komen foundation. https://www.komen.org/breast-cancer/risk-factor/understanding-risks/gail-method/.

[25] WHO model list of essential medicines - 22nd list, 2021. World Health Organization. Sept 30, 2021. https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2021.02.

[26] "Tamoxifen and Breast Cancer." Health Library of Cleveland Clinic. https://my.clevelandclinic.org/health/drugs/9785-tamoxifen.

[27] Breast Cancer Treatment Options-Tamoxifen. Breastcancer.org. https://www.breastcancer.org/treatment/hormonal-therapy/tamoxifen.

[28] Johansson A, Dar H, van't Veer LJ, et al. "Twenty-year benefit from adjuvant goserelin and tamoxifen in premenopausal patients with breast cancer in a controlled randomized clinical trial." Journal of Clinical Oncology, July 21, 2022. https://doi.org/10.1200/JCO.21.02844.

[29] Powles TJ, Hickish T, Kanis JA, Tidy A, Ashley S. "Effect of tamoxifen on bone mineral density measured by dual-energy x-ray absorptiometry in healthy premenopausal and postmenopausal women." Journal of Clinical Oncology, vol. 14, no. 1, 1996, pp. 78-84. http://doi.org/10.1200/JCO.1996.14.1.78.

[30] Gottardis, M.M., Ricchio, M., Satyaswaroop, P.G. and Jordan, V.C. "Effect of steroidal and nonsteroidal antiestrogens on the growth of a tamoxifen-stimulated human endometrial carcinoma (EnCa40) in athymic mice." Cancer Research, vol. 50, no. 11, 1990, pp. 3189-3192.

[31] Gal, D., Kopel, S., Bashevkin, M., Lebowicz, J., Lev, R. and Tancer, L.M. (1991) "Oncogenic potential of tamoxifen on endometria of postmenopausal women with breast cancer-preliminary report." Gynecologic Oncology, vol. 42, no. 2, 1991, pp. 120-123. http://doi.org/10.1016/0090-8258(91)90330-8.

[32] Chang CH, Huang CW, Huang CM, Ou TC, Chen CC, Lu YM. "The duration of endocrine. therapy and breast cancer patients' survival: A nationwide population-based cohort study." Medicine (Baltimore), vol. 98, no. 43, 2019, pp. e17746. http://doi.org/10.1097/MD.0000000000017746.

[33] Davies C, Pan H, Godwin J, et al. for the "Adjuvant Tamoxifen: Longer Against Shorter (ATLAS) Collaborative Group. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial." Lancet, vol. 381, no. 9869, 2013, pp. 805-16. http://doi.org/10.1016/S0140-6736(12)61963-1.

[34] Fleming CA, Heneghan HM, O'Brien D, McCartan DP, McDermott EW, Prichard RS. "Meta-analysis of the cumulative risk of endometrial malignancy and systematic review of endometrial surveillance in extended tamoxifen therapy." British Journal of Surgery. vol. 105, no. 9 Aug 2018, pp. 1098-1106. https://doi.org/10.1002/bjs.10899.

[35] Montoya, M. C. and Krysan, D. J. "Repurposing Estrogen Receptor Antagonists for the Treatment of Infectious Disease." mBio vol. 9, no. 6, 2018, e02272–18. http://doi.org/10.1128/mBio.02272-18.

[36] Brown, AC and Fraser, TR. (1865). "On the Connection between Chemical Constitution and Physiological Action; with special reference to the Physiological Action of the Salts of the Ammonium Bases derived from Strychnia, Brucia, Thebaia, Codeia, Morphia, and Nicotia". Journal of Analytical Physiology, vol. 2, no. 2, 1868, pp. 224-42.

[37] Yang, H., Li, J., Wu, Z., Li, W., Liu, G. and Tang, Y. “Evaluation of Different Methods for Identification of Structural Alerts Using Chemical Ames Mutagenicity Data Set as a Benchmark”, Chemical Research Toxicology, vol. 30, no. 6, 2017, pp. 1355–1364. https://doi.org/10.1021/acs.chemrestox.7b00083.

[38] Hansch, C., Maloney, P., Fujita, T. et al. “Correlation of Biological Activity of Phenoxyacetic Acids with Hammett Substituent Constants and Partition Coefficients.” Nature, vol. 94, 1962, pp. 178–180. https://doi.org/10.1038/194178b0.

[39] FDA Basics. “FDA at a glance.” U.S. Food and Drug Administration. November 2021. https://www.fda.gov/about-fda/fda-basics/fact-sheet-fda-glance.

[40] Pelley, J. “Number of chemicals in commerce has been vastly underestimated-Scientists assemble a first-ever global inventory listing triple the number of chemicals on the market as previous lists.” Chemical and Engineering News, February 12, 2020. https://cen.acs.org/policy/chemical-regulation/Number-chemicals-commerce-vastly-underestimated/98/i7.

[41] McKinney JD, Richard A, Waller C, Newman MC, Gerberick F. “The practice of structure activity relationships (SAR) in toxicology.” Toxicological Sciences, vol. 56, no. 1, 2000, pp. 8-17. http://doi.org/10.1093/toxsci/56.1.8.

[42] Klopman, G and McGonigal, M. “Computer simulation of physical-chemical properties of organic molecules. 1. Molecular system identification.” Journal of Chemical Information and Computer Sciences, vol. 21, no. 1, 1981, pp. 48-52.

[43] Klopman, G. (1984) “Artificial intelligence approach to structure-activity studies. Computer automated structure evaluation of biological activity of organic molecules.” Journal of the American Chemical Society, vol. 106, no. 24, 1984, pp. 7315-7321. http://DOI.org/10.1021/ja00336a004.

[44] Klopman, G.(1933-2015). Multicase, Inc. https://www.multicase.com.

[45] Frierson, M.R., Klopman, G. and Rosenkranz, H.S. (1986) “Structure-activity relationships among mutagens and carcinogens: A review.” Environmental Mutagenesis, vol. 8, no. 2, 1986, pp. 283-327. http://doi.org/10.1002/em.2860080210.

[46] Klopman, G., Wang, S. and Balthasar, D.M. “Estimation of aqueous solubility of organic molecules by the group contribution approach. Application to the study of biodegradation.” Journal of Chemical Information and Computer Science, vol. 32, no. 5, 1992, pp. 474-482. http://DOI.org/10.1021/ci00009a013.

[47] Klopman, G. and Wang, S. “A computer automated structure evaluation (CASE) approach to calculation of partition coefficient.” Journal of Computational Chemistry, vol. 12, no. 8, 1991, pp. 1025-1032. https://doi.org/10.1002/jcc.540120815.

[48] Ames BN, McCann J, Yamasaki E. “Methods for detecting carcinogens and mutagens with the salmonella/mammalian-microsome mutagenicity test.” Mutation Research, vol, 31, no. 6, 1975, pp. 347–364. https://doi.org/10.1016/0165-1161(75)90046-1.

[49] Gold, Lois Swirsky, PhD, Director. “The Carcinogenic Potency Project-The Carcinogenic Potency Database (CPDB).” https://files.toxplanet.com/cpdb/index.html.

[50] Gold LS, Sawyer CB, Magaw R, Backman GM, de Veciana M, Levinson R, Hooper NK, Havender WR, Bernstein L, Peto R, et al. “A Carcinogenic Potency Database of the standardized results of animal bioassays.” Environmental Health Perspectives, vol. 58, 1984, pp. 9-319. http://doi.org/10.1289/ehp.84589.

[51] Gold LS, Slone TH, Manley NB, Garfinkel GB, Hudes ES, Rohrbach L, Ames BN. “The Carcinogenic Potency Database: analyses of 4000 chronic animal cancer experiments published in the general literature and by the U.S. National Cancer Institute/National Toxicology Program.” Environmental Health Perspectives, vol. 96, 1991, pp. 11-15. http://doi.org/10.1289/ehp.919611.

[52] Peto, R., Pike, M.C., Bernstein, L., Gold, L.S. and Ames, B.N. “The TD50: A proposed general convention for the numerical description of the carcinogenic potency of chemicals in chronic-exposure animal experiments.” Environmental Health Perspectives, vol. 58, 1984, pp. 1-8. http://doi.org/10.1289/ehp.84581.

[53] National Toxicology Program. U.S. Department of Health and Human Services. https://ntp.niehs.nih.gov.

[54] Soward, SM, Rowley, RM, Nicholoson, GL and Eastin, WC. “Impact of the World Wide Web on the availability of information from National Toxicology Program databases”. Chemical Health and Safety, vol. 7, no. 4, 2000, pp. 9-18. http://DOI.org/10.1016/S1074-9098(00)00106-4.

[55] Mortelmans K and Zeiger E. “The Ames Salmonella/microsome mutagenicity assay.” Mutation Research, vol. 455, no. 1-2, 2000, pp. 26-60. http://doi.org/10.1016/s0027-5107(00)00064-6.

[56] Ashby, J and Tennant, RW. “Chemical structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTP.” Mutation Research, vol. 204, no. 1, 1988, pp. 17-115. http://doi.org/10.1016/0165-1218(88)90114-0.

[57] Ashby, J and Tennant, RW. “Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP.” Mutation Research, vol. 257, no. 3, 1991, pp. 229-306. http://doi.org/10.1016/0165-1110(91)90003-e.

[58] Kobets, T, Iatropoulos, MJ and Williams, GM. “Mechanisms of DNA-reactive and epigenetic chemical carcinogens: applications to carcinogenicity testing and risk

assessment." Toxicology Research, vol. 8, no. 2, 2019, pp. 123–145. http://doi.org/10.1039/c8tx00250a.

[59] Nohmi, T. "Thresholds of Genotoxic and Non-Genotoxic Carcinogens." Toxicology Research, vol. 34, no. 4, 2018, pp. 281–290. http://doi.org/10.5487/TR.2018.34.4.281.

[60] Stenbygaard, C.E., Herrstedt, J., Thomsen, J.F., Svendsen, K.R., Engelholm, S.A. and Dombernowsky, P. "Toremifene and tamoxifen in advanced breast cancer - a double blind crossover trial." Breast Cancer Research and Treatment, vol. 25, no. 1, 1993, pp. 57-63. http://doi.org/10.1007/BF00662401.

[61] Lim CK, Yuan ZX, Lamb JH, White IN, De Matteis F, Smith LL. "A comparative study of tamoxifen metabolism in female rat, mouse and human liver microsomes." Carcinogenesis, vol. 15, no. 4, 1994, pp. 589-93. http://doi.org/10.1093/carcin/15.4.589.

[62] Ahmad A, Shahabuddin S, Sheikh S, Kale P, Krishnappa M, Rane RC, Ahmad I. "Endoxifen, a new cornerstone of breast cancer therapy: demonstration of safety, tolerability, and systemic bioavailability in healthy human subjects." Clinical Pharmacology and Therapeutics, vol. 88, no. 6, 2010, pp. 814-17. http://doi.org/10.1038/clpt.2010.196.

[63] "DOS Days. Old PC Computing Resource." DOS Day. http://dosdays.co.uk/topics/1994.php.

[64] Lhasa Carcinogenicity Database. A vital source of long-term carcinogenicity study data. Lhasa Limited. https://www.lhasalimited.org/products/lhasa-carcinogenicity-database.htm.

[65] "What we Study." Testing program and Tox21 of the National Toxicology Program. U.S. Department of Health and Human Services. https://ntp.niehs.nih.gov/whatwestudy/index.html.

[66] "The Ames Test or Bacterial Reverse Mutation Test." Europhins Australia-Biopharma product testing. https://www.eurofins.com.au/biopharma-services/genetic-toxicology/the-ames-test/.

[67] Faramarzi, S., Kim, M., Volpe, D., Cross, K., Chakravarti, S., and Stavitskaya, L. "Development of QSAR Models to Predict Blood-Brain Barrier Permeability." Frontiers in Pharmacology, 2022. https://doi.org/10.3389/fphar.2022.1040838.

[68] Chakravarti, S.K., Saiakhov, R.D. "MultiCASE Platform for In Silico Toxicology." In: Benfenati, E. (eds) In Silico Methods for Predicting Drug Toxicity. Methods in Molecular Biology, vol 2425. Humana, New York, NY. pp. 497-518. https://doi.org/10.1007/978-1-0716-1960-5_19.

[69] Stepan, A. F., Walker, D. P., Bauman, J., Price, D. A., Baillie, T. A., Kalgutkar, A. S., and Aleo, M. D. "Structural alert/reactive metabolite concept as applied in medicinal chemistry to mitigate the risk of idiosyncratic drug toxicity: a perspective based on the

critical examination of trends in the top 200 drugs marketed in the United States." Chemical Research in Toxicology, vol. 24, no. 9, 2011, pp. 1345– 1410. http://doi.org/10.1021/tx200168d.

[70] Liu, R., Yu, X., and Wallqvist, A. "Data-driven identification of structural alerts for mitigating the risk of drug-induced human liver injuries" Journal of Cheminformatics, vol. 7, no. 4, 2015. http://doi.org/10.1186/s13321-015-0053-y.

[71] Limban, C., Nuţă, D., Chiriţă, C., Negreș,S., Arsene, A., Goumenou, M., Karakitsios, S., Tsatsakis, A. and Sarigiannis, D. "The use of structural alerts to avoid the toxicity of pharmaceuticals." Toxicology Reports, vol. 5, 2018, pp. 943-953. http://doi.org/10.1016/j.toxrep.2018.08.017.

[72] Benigni, R., Bossa, C., Alivernini, S., and Colafranceschi, M. "Assessment and validation of US EPA's OncoLogic(R) expert system and analysis of its modulating factors for structural alerts." Journal of Environmental Science and Health, Part C. Environmental Carcinogenesis and Ecotoxicology Reviews, vol. 30, no. 2, 2012, pp. 152-173. http://doi.org/10.1080/10590501.2012.681486.

[73] Kruhlak, N.L. "US FDA Experience in the Regulatory Application of (Q)SAR." U.S. Food and Drug Administration. Society of Toxicology - Computational Toxicology Specialty Section Webinar. February 5, 2020. https://www.toxicology.org/groups/ss/CTSS/docs/SOT-CE-Presentation-Kruhlak_final_Updated2.pdf.

[74] ICH Harmonised Guideline for "Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk M7(R1)." International Council for Harmonisation of technical requirements for pharmaceuticals for humans (ICH). Current Step 4 version, March 31, 2017. https://database.ich.org/sites/default/files/M7_R1_Guideline.pdf.

[75] Hinkson, IV, Madej, B and Stahlberg, EA. "Accelerating therapeutics for opportunities in medicine: a paradigm shift in drug discovery." Frontiers in Pharmacology, vol. 11, 2020, pp. 770. https://doi.org/10.3389/fphar.2020.00770.

[76] Matthews RA. "Medical progress depends on animal models - doesn't it?" Journal of the Royal Society of Medicine, vol. 101, no. 2, 2008, pp. 95-98. http://doi.org/10.1258/jrsm.2007.070164.

[77] Palfreyman MG, Charles V, Blander J. "The importance of using human-based models in gene and drug discovery." Drug Discovery World, Fall 2002, pp. 33–40. https://www.ddw-online.com/the-importance-of-using-human-based-models-in-gene-and-drug-discovery-1724-200210/.

[78] Mak, I., Evaniew N. andGhert, M. "Lost in translation: animal models and clinical trials in cancer treatment." American Journal of Translational Research, vol. 6, no. 2, 2014, pp. 114-18.

[79] Hussain, G. “Animals Testing, Exposed: Millions of Animals Suffer in Labs Every Year.” Sentient Media. October 8, 2021. https://sentientmedia.org/animal-testing/.

[80] “About new therapeutic uses.” National Center for Advancing Translational Services. https://ncats.nih.gov/ntu/about.

[81] Gao W, Hu H, Dai L, He M, Yuan H, Zhang H, Liao J, Wen B, Li Y, Palmisano M, Traore MDM, Zhou S, Sun D. “Structure–tissue exposure/selectivity relationship (STR) correlates with clinical efficacy/safety.” Acta Pharmaceutica Sinca B, vol. 12, no. 5, 2022, pp. 2462-2478. http://doi.org/10.1016/j.apsb.2022.02.015.

[82] Ending Cosmetics Animal Testing.” The Humane Society of the United States.” https://www.humanesociety.org/all-our-fights/ending-cosmetics-animal-testing.

[83] “About Animal Testing.” Humane Society International/Global. https://www.hsi.org/news-media/about/.

[84] “IARC Monographs on the identification of carcinogenic hazards to humans.” International Agency on the Research of Cancer (IARC). World Health Organization. https://monographs.iarc.who.int/cards_page/publications-monographs/.

[85] “United States Cancer Statistics: Data Visualizations. " U.S. Cancer Statistics (USCS). Centers for Disease Control and Prevention. https://gis.cdc.gov/Cancer/USCS/#/Trends.

[86] Light, DW. “New Prescription Drugs: A Major Health Risk with Few Offsetting Advantages.” Harvard University - Edmond and Lily Safra Center for Ethics. June 27, 2014. https://ethics.harvard.edu/blog/new-prescription-drugs-major-health-risk-few-offsetting-advantages.