



UNIVERSITI PUTRA MALAYSIA

***FACTORS INFLUENCING AFLATOXIN BIOMARKER LEVEL IN
URINE AMONG ADULTS IN HULU LANGAT DISTRICT, SELANGOR,
MALAYSIA***

SITI HUSNA BINTI SULAIMAN

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By

SITI HUSNA BINTI SULAIMAN

**Thesis submitted to the School of Graduate Studies,
Universiti Putra Malaysia in fulfilment of the requirement for the
Master of Science**

November 2017

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

**FACTORS INFLUENCING AFLATOXIN BIOMARKER LEVEL IN URINE
AMONG ADULTS IN HULU LANGAT DISTRICT, SELANGOR**

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SITI HUSNA BINTI SULAIMAN

November 2017

Chairman : Rosita binti Jamaluddin, Ph.D
Faculty : Medicine and Health Science

Previously, there were several cases related to aflatoxin occurred in Malaysia. Aflatoxin is commonly found in human food resources especially in nuts, cereals, poultry and livestock, and the impact of this food-borne poisoning should be taken seriously. This study involved a total of 468 adults who resided in Hulu Langat district and aged from 18 to 60 years old. Data on socio-demography (SD) knowledge, attitude and practice (KAP) on aflatoxin and its contamination in food, dietary intake (DI), physical activity (PA) and weight status (WS) through the questionnaires and 15ml of non-fasting morning urine samples were collected to measure the aflatoxin level. The quantification of urinary aflatoxin M₁ (AFM₁) was done using ELISA kit. Statistical analysis involved descriptive analysis and inferential analysis.

Of the 468 screened respondents, 444 met the following study criteria: in good health; not taking any medications or supplements; not smoking; not following a restricted diet; not pregnant and not in postpartum period.

There were 249 females and 199 males of which majority of them aged between 18 to 24 years old with an average age of 29.21 years old. More than half of the respondents were single (n= 320) and had RM1500 or less amount of personal income (n=303). There were 238 respondents had low knowledge, 178 respondents had positive attitude while 209 respondents had positive practice towards aflatoxin. A total of 281 respondents had a normal BMI with majority of them (n=190) practised a low level of physical activity. Cereal and cereal-based products recorded the highest food consumption among respondents with an average intake of 512.54g/day. From 444 samples, 199 were positive with AFM₁ with 37 of them exceeded the limit of detection (LOD) of 0.64ng/mL. The statistical analysis showed significant associations in

aflatoxin level based on age group ($p<0.05$), ethnicity ($p<0.01$) and household income ($p<0.01$), attitude ($p<0.01$) and practice ($p=0.03$) towards aflatoxin and its contamination in food, intake of eggs ($p=0.03$) and milk and dairy products ($p<0.01$). Besides, the binomial logistic regression showed ethnicity, household income and intake of dairy products were factors that contributed to aflatoxin occurrence in urine with ethnicity ($W=25.57$, $p<0.01$) as the main predictor. Chinese were 3.20 times more likely to exhibit aflatoxin occurrence in urine compared to non-Chinese.

When both husband and wife are working, the household income increases and encourages the eating-out activity. Eating-out exposes consumers to aflatoxin contamination, as not all food premise owners care about the presence and danger of this toxin to humans. Chinese were having the highest levels of urinary aflatoxin due to high intake of potentially aflatoxin-contaminated foods such as cereals, nuts, milk and eggs. This research was one of the first steps in increasing the awareness towards aflatoxin among adults in Hulu Langat district, Selangor. Findings from this research showed the current level of aflatoxin among Malaysians in Hulu Langat and the factors that might influence the aflatoxin presence in human based on the aflatoxin biomarker level.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

**FAKTOR YANG MEMPENGARUHI PARAS BIOMARKER AFLATOKSIN
DALAM URIN DI KALANGAN ORANG DEWASA DI DAERAH HULU
LANGAT, SELANGOR**

Oleh

SITI HUSNA BINTI SULAIMAN

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Sebelum ini, terdapat beberapa kes yang berkaitan dengan aflatoksin berlaku di Malaysia disebabkan pengambilan makanan yang tercemar dengan aflatoksin. Aflatoksin lazimnya didapati di dalam sumber makanan manusia terutamanya dalam kacang, bijirin, ayam dan ternakan maka kesan keracunannya perlu diberi perhatian serius. Kajian ini melibatkan 468 orang dewasa yang menetap di Hulu Langat serta berusia antara 18 hingga 60 tahun. Data berkaitan latar belakang (SD), pengetahuan, sikap dan amalan terhadap aflatoksin (KAP), pengambilan makanan (DI), aktiviti fizikal (PA) dan status berat (WS) diperolehi melalui satu soal selidik dan 15ml sampel urin tidak berpuasa dikumpul untuk mengukur tahap aflatoksin. Pengukuran aflatoksin M₁ (AFM₁) di dalam air kencing dilakukan dengan menggunakan kit ELISA. Analisis statistik melibatkan analisis deskriptif dan analisis inferensi.

Daripada 468 responden yang terlibat, 444 memenuhi kriteria berikut: mempunyai kesihatan yang baik; tidak mengambil apa-apa ubat atau makanan tambahan; tidak merokok; tidak mempunyai diet yang tertentu; tidak hamil dan bukan dalam tempoh berpantang.

Terdapat 249 wanita dan 199 lelaki terlibat dalam kajian ini di mana majoriti daripada mereka yang berusia antara 18 hingga 24 tahun dengan purata usia 29.21 tahun. Lebih separuh daripada responden berstatus bujang (n=320) dan mempunyai pendapatan peribadi berjumlah RM1500 dan kurang (n=303). Sebanyak 238 responden mempunyai pengetahuan yang rendah terhadap aflatoksin 178 orang responden mempunyai sikap yang baik terhadap aflatoksin manakala 209 orang responden mempunyai amalan yang baik terhadap aflatoksin. Sejumlah 281 responden mempunyai BMI yang normal

dengan majoritinya ($n=190$) mengamalkan aktiviti fizikal bertahap rendah. Bijirin dan produk berasaskan bijirin mencatatkan pengambilan makanan yang tertinggi di kalangan responden dengan purata pengambilan sebanyak 512.54kg/hari. Daripada 44 sampel, 199 adalah positif AFM₁ dengan 37 daripadanya melebihi had pengesanan (LOD), 0.64ng/mL. Hasil analisis mendapati terdapat kaitan yang signifikan antara tahap aflatoxin dengan kategori umur ($p<0.05$), etnik ($p<0.01$), pendapatan isi rumah ($p<0.01$), amalan ($p<0.01$) dan sikap ($p=0.03$) terhadap aflatoxin dan pencemarannya dalam makanan, pengambilan telur ($p=0.03$) dan juga susu dan produk tenusu ($p<0.01$). Selain itu, regresi logistik binomial menunjukkan etnik, pendapatan isi rumah serta pengambilan susu dan produk tenusu merupakan faktor penyumbang kepada berlakunya aflatoxin dalam air kencing, dengan etnisiti ($W=25.57$, $p<0.01$) sebagai peramal utama di kalangan semua. Kaum Cina didapati 3.20 kali lebih cenderung mempunyai aflatoxin biomarker di dalam air kencing berbanding dengan kaum lain.

Apabila kedua suami isteri bekerja, pendapatan isi rumah meningkat dan menggalakan mereka untuk makan di luar. Pengambilan makanan luar mendedahkan pengguna kepada pencemaran aflatoxin memandangkan tidak semua pemilik premis makanan peduli dengan kehadiran dan bahayanya toksin tersebut kepada manusia. Kaum Cina didapati mempunyai paras aflatoxin yang tertinggi di dalam urin dan ini berkaitan dengan pengambilan makanan-makanan yang berpotensi terdedah dengan aflatoxin seperti bijirin, kacang, susu dan telur. Kajian ini adalah salah satu daripada langkah-langkah pertama dalam meningkatkan kesedaran terhadap aflatoxin di kalangan orang dewasa di kawasan Hulu Langat, Selangor. Penemuan daripada kajian ini menunjukkan tahap semasa aflatoxin di kalangan rakyat Malaysia di Hulu Langat dan mengenalpasti faktor-faktor yang mungkin mempengaruhi kehadiran aflatoxin dalam manusia berdasarkan tahap biomarker aflatoxin.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows

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LIST OF ABBREVIATIONS

AFB ₁	Aflatoxin B ₁
AFB ₂	Aflatoxin B ₂
AFG ₁	Aflatoxin G ₁
AFG ₂	Aflatoxin G ₂
Aflatoxin M ₁	AFM ₁
Aflatoxin M ₂	AFM ₂
Aflatoxin P ₁	AFP ₁
Aflatoxin Q ₁	AFQ ₁
AFM ₁	Aflatoxin M ₁
BMI	Body mass index
CAC	Codex Alimentarius Commission
DI	Dietary intake
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
FFQ	Food frequency questionnaire
FTC	Food Technology Center
GC	Gas chromatography
GP-IPS	Geran Putra-Inisiatif Putra Siswazah
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HPLC	High pressure liquid chromatography
IARC	International Agency for Research on Cancer
IMR	Institute of Medical Research
IPAQ	International Physical Activity Questionnaire
KAP	Knowledge, attitude, practice
LOD	Level of detection
MARDI	Malaysian Agricultural Research & Development Institute
MOH	Ministry of Health
MPKJ	Majlis Perbandaran Kajang
PA	Physical activity
RMC	Research Management Centre
SCF	Scientific Committee on Food
SD	Socio-demography
TLC	Thin layer chromatography
UPM	Universiti Putra Malaysia
WHO	World Health Organization
WS	Weight status

CHAPTER 1

INTRODUCTION

1.1 Background

Responsibility concerning food safety is necessary among people to overcome food-borne illnesses and poisonings. However, the level of awareness among people around the world is still below satisfactory level as cases related to food-borne illnesses and poisonings are still reported all over the world (Diane *et al.*, 2010). As the food is exposed to hazardous agents biologically, physically or chemically, the contaminated food may lead to adverse health consequences towards human and animals. These hazardous agents may be introduced into food and feed via harvesting, formulation and processing, packaging and labelling, storage, preparation, transportation and serving. There are a variety of chemical hazards involved in the food industry, but the most notable ones are mycotoxins, natural and marine toxins, food additives, agricultural products and veterinary drug residues. The most emphasized chemical hazard that will be discussed here is mycotoxin.

Mycotoxins are natural toxicants of moulds and fungi. These fungal secondary metabolites are found everywhere and unavoidable within the natural world. The growth of moulds and fungi is normally promoted by certain temperature with the presence of moisture. They are produced by filamentous molds especially from the family of *Fusarium*, *Penicillium* and *Aspergillus* sp. (Pitt *et al.*, 2000). Even though all mycotoxins originated from fungi, but, not all toxic compounds produced by fungi are termed as mycotoxins. The role of metabolite and its function to living things are the conditions that should be seen in terming the fungi-toxic compounds (Bennet and Klich, 2003). When the toxic compounds are fatal to bacteria, the toxin is called antibiotics, otherwise when the toxic compounds are fatal to plants, the plant pathologists termed them as phytotoxins. The one that is toxic to human and animals is called as mycotoxins. The most recognized mycotoxins presence in food and feeds are aflatoxins, fumonisins, ochratoxins, trichothecenes, zearalenone and patulin (Marinet *et al.*, 2013).

Mycotoxicosis is a disease that leads to chemical contamination via food and feed which is associated with the exposures to mycotoxins produced by fungi (Robert *et al.*, 2010). Poor storage and production process of grain and feed for poultry attribute the growth of toxin-producing fungi which results in the production of mycotoxin (Mohamed, 2011). There are a lot of mycotoxicosis cases which happened globally due to fungal infections and some of them were initially reported to occur widely in the European countries in 1960s (Asao *et al.*, 1963). The infection of fungi in diets caused the outbreak of disease known as St. Anthony's or Holy Fire in many European countries due to the contamination of rye by ergot alkaloid, produced by *Claviceps purpurea*

(Paterson and Lima, 2010). Since then, many cases have been reported and the discovery of "Turkey X" disease in London, England caused by aflatoxin in 1950s and early 1960s had opened new prospectus on the scientific research related to the aetiology of mycotoxicosis and preventive strategies that exists in foods, animals and human (Kensler *et al.*, 2011). Kensler *et al.* (2011) described the epidemic disease as the major cause of death of numerous poultry animals due to the consumption of feeds containing nuts and cereals, contaminated by *Aspergillus flavus*, a pathogenic fungus. It was found that extracts from the culture of the fungus isolated from the meal were found to have the capability to induce "Turkey X" syndromes (Kensler *et al.*, 2011).

The same situation is faced by Malaysians as evident by recent publications and reports on the occurrences of contaminants and toxicants in the diets and their impacts on human and animals (Leong *et al.*, 2010; Arzandeh *et al.*, 2010; Sulaiman *et al.*, 2007). There was a report in Malaysia about mycotoxicosis, a food-borne poisoning, namely aflatoxicosis. Back in 1988, this case has led to thirteen deaths of children in Perak (Lye *et al.*, 1995). This aflatoxicosis case appeared due to the presence of aflatoxin, a mycotoxin by fungi which is commonly found in human food resources especially in nuts and cereals and poultry and the impact of this food-borne poisoning should be taken seriously. The tropical climate prevailing in Malaysia accompanied with poor storage and production process enhance the growth of *Aspergillus* species of fungi and subsequently the production of aflatoxin in the foodstuffs, in which can lead to the occurrence of aflatoxicosis.

There are a large number of mycotoxins found in human and animal's diet. Aflatoxins, trichothecenes, ochratoxins, fumonisins and zearalenone are the most important mycotoxins in food and feed industries with aflatoxins as the most studied mycotoxin among all. Aflatoxins are classified into several types according to their fluorescent properties under ultraviolet light. Of many aflatoxin types, aflatoxin B₁ (AFB₁) is classified by the International Agency for Research on Cancer (IARC) as group 1 carcinogen. In fact, AFB₁ owing to its mutagenic and carcinogenic properties has been classified as a major risk factor, alongside the hepatitis B virus (HBV) and the hepatitis C virus (HCV) (Kensler *et al.*, 2011) for the development of liver cancer.

1.2 Problem Statement

Aflatoxin is classified as hepatotoxic, mutagenic, carcinogenic difuran-containing, polyketide-derived *Aspergillus* toxin and aflatoxin B₁ (AFB₁) has been classified by International Agency for Research on Cancer (IARC) as the most carcinogenic aflatoxin. Liver is the main organ for metabolizing aflatoxin in human and animals (Monosson, 2012) but aflatoxin metabolizing process may occur directly during absorption, in the blood or in several hepatic organs. Hence, aflatoxin exposure can cause liver cancer and its chronic exposure disturbs the immune system (Jiang *et al.*, 2005; Meissonier *et al.*, 2006) in human and interferes protein synthesis in animals (Applegate *et al.*, 2009).

Many people do not recognize the exposure until the critical and chronic symptoms and signs appear. To overcome this problem, early stage of prevention must be conducted. Before the preventive measures are taken, the detection of aflatoxin in the biological samples needs to be made as these biomarkers are feasible, reliable and reflect the exposure to aflatoxin through the diet. The prevalence and level of human exposure to aflatoxin together with its contributing factors are limitedly discovered and thus, more studies on aflatoxin exposure should be carried out to discover those possible factors. Even though there are almost similar studies conducted (Leong *et al.*, 2012; Mohd Redzwan *et al.*, 2012), these data cannot be used to represent the general aflatoxin exposure in Malaysia. As the main route of aflatoxin exposure is through the diet, it is possible that different populations are exposed to different aflatoxin exposure. This has been proven by a previous study conducted in Penang by Leong and colleagues (2012). They discovered a high level of aflatoxin among Chinese respondents with 3.05 times higher than Malay respondents. In fact, the use of aflatoxin biomarkers to assess human exposure to aflatoxin in Malaysia is still in its infancy stage (Mohd Redzwan *et al.*, 2013). Thus, this study is carried out to provide additional information towards the extent of human exposure to aflatoxin in different population; that is in Hulu Langat and to find the underlying factors for the detection of aflatoxin biomarker.

1.3 Research Questions

By taking into account the stated problems above, this study was conducted to discover the possible factors that contributed to the presence of aflatoxin AFM₁ biomarker in urine among healthy adults in Hulu Langat district, Selangor. Hence, this study was conducted to answer:

- a. What is the level of aflatoxin among adults in Hulu Langat?
- b. Is there any association in aflatoxin AFM₁ biomarker level among adults in Hulu Langat district based on socio-demographic (SD) variables (genders, age, marital status, ethnicity, educational level, personal and household income)?
- c. Are there any associations between knowledge, attitude and practice (KAP) towards aflatoxin, physical activity (PA) level, dietary intake (DI), and weight status (WS) towards aflatoxin AFM₁ biomarker level among adults in Hulu Langat district?
- d. What are the factors influencing the presence of aflatoxin AFM₁ biomarker among adults in Hulu Langat district?

1.4 Objectives

This study is conducted to determine the prevalence and factors influencing aflatoxin presence in urine (AFM₁) among healthy adults in Hulu Langat district, Selangor.

1.4.1 Specific Objectives

1. To measure aflatoxin level among healthy adults in Hulu Langat district based on aflatoxin AFM₁ biomarker in urine samples.
2. To determine the associations between socio-demographic (SD) characteristics and aflatoxin AFM₁ biomarker level in urine.
3. To determine the associations between knowledge, attitude and practice scores, physical activity level, dietary intake and weight status of participants with detectable aflatoxin AFM₁ biomarker level in urine.
4. To evaluate the predictors to the presence of aflatoxin biomarker, AFM₁ in urine samples among adults in Hulu Langat district.

1.5 Hypothesis

There are four null hypotheses to be tested in this study:

H₀1: There are no association between socio-demographic characteristics with aflatoxin AFM₁ biomarker level.

H₀2: There is no association between knowledge, attitude and practice with AFM₁ biomarker level.

H₀3: There is no association between dietary intakes, physical activity and weight status with AFM₁ biomarker level.

H₀4: The chosen factors like socio-demography variables (gender, age, marital status, ethnicity, educational level, personal income and household income), knowledge, attitude and practice, dietary intake, physical activity level and weight status are not the predictors in aflatoxin AFM₁ biomarker presence.

1.6 Significance of Study

Aflatoxin is an acute cancer-causing chemical which is commonly found in staple commodities such as cereals and cereal-based products which are stored improperly. Once contaminated, the aflatoxin will remain in the food even after being processed. This harms both animals (pet and agricultural animals) and human. In animals, the ingested aflatoxin will be transformed into its by-products and are found in eggs, milk products and meat (Fratamico *et al.*, 2008). This has been proven by a study in Pakistan which showed detectable aflatoxin biomarkers in chicken meats and eggs after the chickens were fed on the aflatoxin-contaminated feeds (Shahzad *et al.*, 2014).

Even though aflatoxicosis cases rarely reported in Malaysia and majority of Malaysians were not concerned on the possible adverse effect of it, they still need to be alert on the consequences of the long-term exposure of this toxin. Aflatoxins, especially the B₁ type affects the liver as it is the main biochemical synthesizer and detoxifying organ of many toxicants. Excessive dietary aflatoxins exposure is one of the causes to hepatic diseases such as cirrhosis and carcinoma in liver as reported in Asia and Africa (Cristina *et al.*, 2014). There is no specific antidote discovered yet to overcome aflatoxicosis. Thus,

people need to take the first step in creating the awareness towards aflatoxin and control its exposure from entering their body.

Therefore, this research was one of the first steps in creating the awareness towards aflatoxin among adults in selected areas in Hulu Langat, Selangor. Findings from this research will be able to show the current level of aflatoxin among Malaysians in Hulu Langat and the factors that might influence the aflatoxin presence in human based on the aflatoxin biomarker level. Then, these findings may alert Malaysians by giving information and guideline in reducing the risk for aflatoxin exposure among Malaysians, due to their varieties of lifestyles and multi-ethnicity population.

1.7 Conceptual Framework

Conceptual framework in **Figure 1.2** illustrates the connection of independent and dependent variables. The chosen factors such as socio-demography characteristics, level of knowledge, attitude and practice towards aflatoxin, dietary intake of possible aflatoxin-contaminated food, physical activity level and weight status were measured in this study to determine the association between them onto AFM₁ biomarker level among adults in selected area in Hulu Langat district, Selangor.

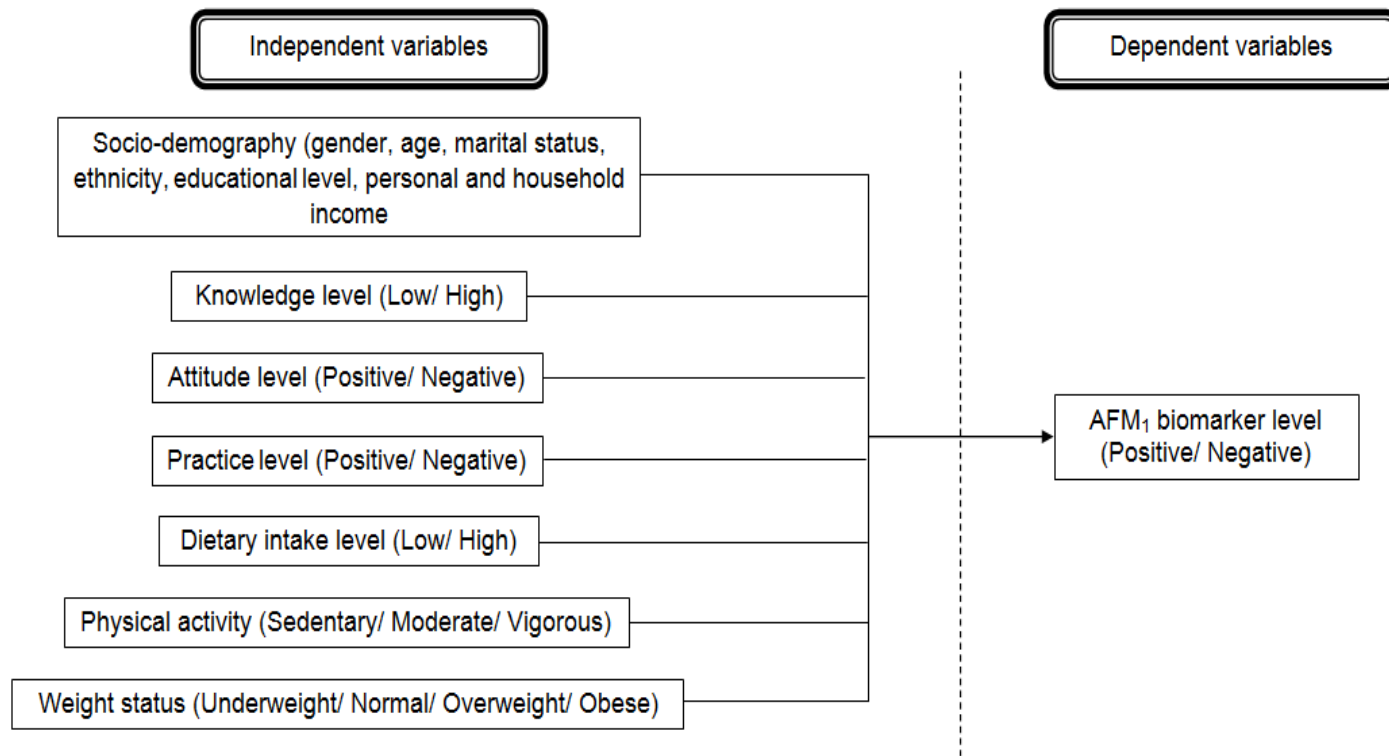


Figure 1.1: Conceptual framework

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