TOXIC EFFECTS ON MODEL MAMMALIAN CELLS BY MONOHALOGENATED DISINFECTION BYPRODUCTS: HALOACETIC ACIDS AND HALOACETONITRILES

BY

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DISSERTATION

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ABSTRACTS

Disinfection processes provide a necessary barrier against waterborne infectious diseases; yet, water disinfectants inadvertently generate environmentally hazardous chemicals called disinfection by-products (DBPs). While a number of individual DBPs have been found to be mutagenic, cytotoxic, genotoxic, carcinogenic, teratogenic and developmentally toxic, the precise mechanisms of toxic action by DBPs is largely unknown. With the recent findings of toxicity pattern associated with halogen and nitrogenous functional groups, haloacetic acids (HAAs) and haloacetonitriles (HANs) were selected as representative carbonaceous and nitrogenous DBPs to make comparisons in terms of genomic DNA damage induction, repair efficiency of induced DNA lesions, and cell cycle alteration using Chinese hamster ovary (CHO) cells as a model mammalian cell system. Both HAAs and HANs are alkylating agents with a halogen atom attached on their carbon atom next to the functional group. The potency of cytotoxicity and genotoxicity induction by monoHAAs and monoHANs is associated with the propensity of the halide to leave the molecule, iodide > bromide > chloride.

The microplate-based single cell gel electrophoresis assay was modified to include liquid holding recovery time to measure the reduction of genomic DNA damage level over time. The repair efficiency for monoHAA-induced lesions by CHO cells followed the rank order of iodoacetic acid > chloroacetic acid >> bromoacetic acid. MonoHAN-induced lesions were repaired more efficiently than monoHAA-induced lesions without consistent significant associations among the three monoHANs. Consequently, the contribution of the halogen atom did not correlate well with DNA repair kinetics.

HAN treatment followed by the release from the treatment resulted in CHO cells with abnormally high levels of DNA content, which was not observed in HAA-treated cells. It appeared that HANs adversely affected cell cycle progression by interfering with cell division. Altered cell ploidy affects genome instability and is widely associated with cancer progression. HAN-mediated disruption of the cell cycle and the generation of aberrant aneuploid cells may contribute to cancer induction and to adverse

pregnancy outcomes associated with consumption of disinfected water observed in epidemiological studies. This is the first study that identifies significant cell cycle alterations by a class of DBPs.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Chemical disinfection has provided protection against microbiological contamination and greatly suppressed outbreaks of waterborne diseases [1]. In the United States the first use of chlorine for disinfection goes back to 1908 in New Jersey [2]. In water works practice, disinfection aims to inactivate pathogenic organisms in the water (primary disinfection) and to maintain a disinfectant residual in the treated water throughout the distribution system to prevent bacterial re-growth (secondary disinfection). Chemical disinfectants are effective for inactivating pathogenic organisms, however, with their high oxidizing ability they also react with natural organic matter (NOM), anthropogenic contaminants and bromide/iodide to form environmentally hazardous chemicals called disinfection byproducts (DBPs) [3, 4]. DBP formation is affected by NOM concentration, pH, temperature, disinfectant concentration and reaction time [5, 6]. Commonly used chemical disinfection agents include free chlorine, combined chlorine (also known as chloramines), chlorine dioxide and ozone, and each of them produces DBPs with different chemical class distributions [7, 8]. Since the first discovery of DBPs in 1974 [9, 10], significant efforts were expended to elucidate DBP formation, occurrence and health effects. To date more than six hundred DBPs have been identified; the most abundant chemical class of DBP in chlorinated water is trihalomethanes (THMs), followed by haloacetic acids (HAAs) [11].

Rook, a Dutch water chemist, reported the formation of chloroform and the other THMs in chlorinated drinking water and showed that the THMs were produced by reactions between chlorine and NOM in raw surface waters [9]. After the National Cancer Institute (NCI) published results of a rodent cancer bioassay on chloroform in 1976 [12], health concerns associated with chloroform and THMs rapidly lead to the adoption of drinking water guidelines and standards to control DBPs and to minimize consumers' exposure to these potentially hazardous chemicals. In 1979, the U.S. Environmental Protection Agency (U.S. EPA) issued the Total Trihalomethane Rule under the Safe Drinking Water Act

with a regulatory standard for THM4 (sum of chloroform, bromodichloromethane, chlorodibromomethane, and bromoform) at 100 µg/L as a running annual average of four quarterly samples [13]. The U.S. EPA published the final Stage 1 Disinfectants/Disinfection By-Product (D/DBP) Rule in 1998 with the lowered THM4 maximum contaminant level (MCL) to be 80 µg/L. The Stage 1 D/DBP Rule established a new MCL of 60 µg/L for the sum of five of the haloacetic acids (HAA5), as well as for bromate (10 µg/L) and chlorate (1.0 mg/L). Thereafter, the final Stage 2 D/DBP Rule was published in 2006. The Stage 2 D/DBP Rule maintains the same numeric MCLs from the Stage 1, but adds modifications to compliance monitoring design and compliance determinations [14, 15].

Epidemiology studies and laboratory animal studies were conducted to determine whether exposure to these agents in drinking water increases human health risks, particularly reproductive and developmental effects and cancer. Toxicological studies demonstrated that certain DBPs cause cancer in the liver, kidney and/or large intestine of laboratory animals and that particular DBPs cause adverse reproductive or developmental effects [16, 17]. On the other hand, epidemiological studies associated increased risk for bladder, colon and rectal cancers with long-term consumption of chlorinated surface waters [18]. Other epidemiological studies indicated adverse reproductive or developmental health effects, such as spontaneous abortion or fetal abnormalities [19, 20]. Therefore, animal toxicology studies have failed to demonstrate the induction of cancer at the sites indicated by epidemiological studies [17]. None of the DBPs identified are plausible bladder cancer carcinogens and they cannot account for the cancer risks projected from epidemiological studies [21].

There are several factors that could explain the discrepancies in the target organ of cancer between epidemiological studies and rodent single chemical bioassays. Most bioassays on DBPs cannot account for the effect of genetic factors and health status, which is likely to be much greater in heterogeneous human populations than in laboratory animals [17]. Also there is an effect from exposure to multiple DBPs, which could interact additively or synergistically [22]. A large percentage of DBPs have not been identified [11] and most of the identified DBPs have not been adequately evaluated for their toxicity or carcinogenicity [23].

The toxic properties of DBPs have been studied for over the past 30 years, yet a systematic quantitative evaluation of DBPs was lacking until recently [23]. The most complete systematic DBP toxicity database consists of >70 DBPs analyzed for chronic cytotoxicity and acute genotoxicity in Chinese hamster ovary (CHO) cells, which made their toxic potency comparable across various chemical classes [24]. From this DBP database, the impact of halogen atom on toxicity within a set of matched compounds was determined. When the combined cytotoxicity and genotoxicity index values were calculated for a set of 18 DBPs with matched halogen analogues, and were averaged as a function of the halogen species, iodinated DBPs were demonstrated to be substantially more cytotoxic and genotoxic than their brominated and chlorinated analogues [24]. Comparison between 26 carbonaceous DBPs (C-DBPs; defined as those not containing nitrogen) and 29 nitrogenous DBPs (N-DBPs; DBPs containing nitrogen) showed that the N-DBPs were substantially more cytotoxic and genotoxic than C-DBPs [24]. It is worth noting that currently there are no U.S. EPA-regulated N-DBPs or iodinated DBPs.

Due to the more stringent Stage 2 D/DBP Rule, many U.S. utilities have switched, or are switching from free chlorine to combined chlorine since chloramination significantly reduces the formation of regulated DBPs [25]. In the U.S. EPA nationwide occurrence study, iodinated THM yield was highest with chloramination disinfection systems, in particular for those plants that did not use ozone or chlorine as a pre-oxidant [4]. Detection of the higher level of iodinated THM in chloramination systems raised the concern of adverse public health consequences [23]. Contributions from treated wastewater discharge and algal activity in drinking water supplies can lead to elevated levels of dissolved organic nitrogen (DON), which may promote formation of emerging N-DBPs of health concern (e.g. haloacetonitriles, nitrosamines) [26-32].

The research in this dissertation focuses on two chemical classes of DBPs; the haloacetic acids (HAAs) and haloacetonitriles (HANs) as representative C-DBPs and N-DBPs. Both chemical classes have relatively high occurrence levels in finished water, although HAAs generally form at much higher concentrations (Table 1.1). HAAs and HANs are both alkylating agents with one or more halogen atoms attached on their carbon atom (α-carbon) next to the functional group (Figure 1.1, 1.2). In both HAAs and

HANs, dihalogenated and trihalogenated species are generally more prevalent; in a survey of 12 U.S. drinking water treatment plants, the median levels of dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) were 15 µg/L and 5.8 µg/L, respectively, while monochloroacetic acid (CAA) was 5.5 µg/L at the maximum. Likewise, the median levels of dichloroacetonitrile (DCAN) and bromochloroacetonitrile (BCAN) were 0.7 µg/L and 0.8 µg/L, respectively, while the median level of chloroacetonitrile (CAN) was 0.05 µg/L (Table 1.1) [11]. The primary interest of this study is to make a comparison focusing on the toxicity pattern associated with halogen and nitrogenous functional groups, thus, monohalogenated species were selected as model compounds. Toxic effects of these selected DBP were compared in terms of genomic DNA damage induction, repair efficiency of induced DNA lesions, and cell cycle alteration using CHO cell as a model mammalian cell.

1.2 Haloacetic Acids

HAAs are detected in finished drinking water [11] and chlorinated effluents from wastewater treatment plants [33] as well as in recreational pool waters [34]. In chlorinated water, HAAs are the second most common class of DBPs [11] (Figure 1.1). The sum of five HAAs (monochloro-, dichloro-, trichloro-, monobromo- and dibromoacetic acid), or HAA5, is regulated at a maximum contaminant level of 60 µg/L [14, 35]. All five regulated HAAs were mutagenic in bacteria [36] and induced genomic DNA damage in mammalian cells *in vitro* [37, 38]. A comparative systematic analysis of chronic cytotoxicity and acute genomic DNA damaging capacity of 12 individual HAAs in mammalian cells was reported [39]. Some of the HAAs are developmentally toxic [40-42] but not clastogenic in human TK6 cells [43]. Carcinogenicity has been shown for DCAA, TCAA, bromochloroacetic acid (BCAA) and dibromoacetic acid (DBAA), but not for CAA [17, 44-46]. Recently the tumorgenicity of iodoacetic acid (IAA) was established [47].

MonoHAA has a single halogen substituent at the α-carbon, which can undergo S_{N2} substitution reaction. The association of toxic potency and halogen substitution was first realized by Richard and Hunter, with the rank order of IAA > bromoacetic acid (BAA) > CAA > fluoroacetic acid for their

developmental toxicity [42]. Later, the level of toxic potency was shown to follow the rank order of IAA > BAA > CAA in *Salmonella typhimurium* mutagenicity [36], forward mutation in Chinese hamster ovary (CHO) cells [48], and DNA damage induction in CHO cells [37] and human cells [49]. This universal pattern of rank order of IAA > BAA > CAA was associated with the inhibition of glyceraldehyde-3-phosphate dehydrogenase activity [50, 51], a first demonstration of molecular mechanisms of toxic action by haloacetic acid DBPs.

1.3 Haloacetonitriles

Haloacetonitriles consists of a cyano group attached to the α-carbon atom with halogen substitution (Figure 1.2). Both α-carbon and cyano carbon can serve as a reactive center [52]. HANs are not currently regulated by U.S. EPA, but the World Health Organization set the guideline value for dichloroacetonitrile (DCAN) and dibromoacetonitrile (DBAN) at 20 µg/L and 70 µg/L, respectively [53] (http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/). The major species of HANs are DCAN, trichloroacetonitrile (TCAN), DBAN and bromochloroacetonitrile (BCAN) and these species are sometimes referred as HAN4. Occurrence and toxicological studies often center around these four species.

Early in the 1980s dihaloacetonitriles were quantified in chlorinated natural waters [54]. The research activity into nitrogenous DBPs (N-DBPs), in which HANs are categorized, has increased partly because water providers are increasingly treating water sources which are impacted by algae blooms and municipal wastewater plant effluent discharge, both key sources of dissolved organic nitrogen (DON) that serve as N-DBP precursors. Population growth and rising water use is likely to make this issue more prevalent during the remainder of this century [31]. Also, recent mammalian cell tests have shown that HANs, haloacetamide and halonitromethanes are all far more cyto- and genotoxic than the regulated THMs and HAAs [24, 55, 56]. Many reactive N-DBP precursors are amino acids or amines, which are well-studied sub-categories of organic matter [31]. DCAN was shown to form from chlorination of aspartic acid and tryptophan with the yield of 0.06 mol/(mol precursor) and 0.09 mol/(mol precursor), respectively [57]. Trehy et al. also showed that aspartic acid produces 0.69 (mol DCAN)/(mol precursor)

at pH 6.4 [58]. DCAN can form from the nitrogen rich fraction of organic matter with high yield [29]. However, it is worth noting that these laboratory-based formation potential tests tend to overestimate the conversion yield compared to actual water treatment plants since formation potential tests are designed to maximize DBP formation.

Nationwide occurrence studies showed that the concentration for the sum of 9 species of chlorinated and/or brominated HANs in finished water from 12 U.S. water treatment plants was from non-detectable to 14 µg/L (median: 3 µg/L) [11]. An occurrence study of effluents from 23 U.S. municipal wastewater treatment plants measured the HAN concentration from 0.9 to 30 µg/L (median: 16 µg/L) in chlorinated effluents and non-detectable to 12 µg/L (median: 0.3 µg/L) in chloraminated effluents [33]. Recently HANs were shown to form as an electrochemical oxidation by-product of landfill leachate; DCAN and BCAN constituted 19-25% of the total concentration of organo-chlorinated compounds [59], especially DCAN at the concentration of 104 – 424 µg/L. HAN formation in biological systems *in vivo* from residual hypochlorite in drinking water was also reported [60].

Salmonella typhimurium mutagenicity was reported for chloroacetonitrile (CAN), DCAN, trichloroacetonitrile (TCAN), and BCAN without metabolic activation, and CAN and bromoacetonitrile (BAN) with metabolic activation [61-63]. CAN, DCAN, TCAN, BAN and DBAN induced DNA damage in HeLa S3 cells [61] and sister chromatid exchange in Chinese hamster ovary (CHO) cells [63]. Clastogenicity of HANs was observed in *Pleurodeles waltl* larvae [62] but not in polychromatic erythrocytes in CD-1 mice [63]. The alkylating potential of the HANs followed the order DBAN > BCAN > CAN > DCAN > TCAN in a cell-free reaction system while the rank order of producing DNA strand breaks in cultured human lymphoblastic cells was TCAN > BCAN > DBAN > DCAN > CAN [64, 65]. HANs reacted toward calf thymus DNA *in vitro* with the reactivity rank order of BAN > CAN > DCAN > TCAN to form 7-(cyanomethyl)guanine [66]. When administered orally to rats, the HANs were metabolized to cyanide and excreted in the urine as thiocyanate. The extent of thiocyanate excretion was CAN > BCAN > DCAN > DBAN >> TCAN [65]. DCAN, but not DBAN, was shown to be an effective

inducer of aneuploidy in oocytes of *Drosophila melanogaster*; the potency of nitriles was attributed to cyanide toxicity [67].

DBAN, BCAN, and CAN were tumor initiators in the mouse skin with topical applications [63], but DBAN is the only haloacetonitrile that has been adequately studied for carcinogenic potential in laboratory animals [17, 68]. In rats, increased incidences of neoplasm were observed in the oral cavity and granular stomach, whereas in mice, increased incidences of squamous cell neoplasms were observed in the forestomach [68]. Based on the carcinogenicity, quantitative structure toxicity relationship model calculated the contribution of DBAN to lifetime risk of cancer to be approximately 3 – 10× more potent than the regulated THMs [21].

Several cases of developmental toxicity were reported for HANs. Treatment of Long-Evans rats with TCAN and DCAN resulted in reduced fertility and increased early implantation failure [69]. DCAN caused soft tissue and skeletal malformation in the surviving fetuses when DCAN was dosed to pregnant Long-Evans rats [70]. Ahmed et al. indicated that fetal brain update of CAN/metabolites induced oxidative stress that lead to increased apoptotic neural cell death in developing mouse brain [71]. Intrauterine exposure to low levels of CAN decreased fetal body weight and induced malformations in the musculoskeletal system in mice [72].

Although there are numerous toxicology studies on HANs, a systematic comparative toxicology study including iodoacetonitrile (IAN) was not published until 2007 by Muellner et al. [55]. Genotoxicity and cytotoxicity of HANs using CHO cells were rank ordered as DBAN > BAN ≈ IAN > BCAN > DCAN > CAN > TCAN for chronic cytotoxicity, and IAN > BAN ≈ DBAN > BCAN > CAN > TCAN > DCAN for acute genotoxicity [55].

1.4 Rationale for Research

Association of toxic potency with halogen substitution pattern was found in mouse whole embryo culture, with the rank order of IAA > BAA > CAA [42]. Subsequently, the same pattern was found in bacteria [36], CHO cells [37, 48] and human cells [49]. However, the repair efficiency of monoHAA-

induced lesions followed the different rank order of IAA > CAA > BAA [73]. These data initiated a question: would different halogen substitution result in different level of DNA lesions? Biological systems have DNA repair capacity for DNA damage to maintain genomic integrity and stability. Basic DNA repair mechanisms include direct DNA repair, base excision repair, nucleotide repair, mismatch repair, homologous recombination repair and non-homologous end joining [74]. Different rates of genomic DNA repair should indicate different DNA lesions and/or different distributions of DNA lesions. Thus, it would be interesting to assess if a different rank order in DNA repair kinetics would be observed with a class of N-DBPs, which is discussed in Chapter 3. Single cell gel electrophoresis assay (Figures 1.3 and 1.4) was modified to include liquid holding recovery time to measure the repair rate of genomic DNA damage (Figure 1.5).

DNA damage induction, DNA damage repair, and cell cycle regulation are strongly associated. In response to DNA damage, cells can activate checkpoints at critical stages in the cell cycle that prevent cells from progressing through the cell cycle [74, 75]. Attene-Ramos et al. [49] conducted a human toxicogenomic analysis of monoHAAs focusing on DNA damage signaling pathways. Transcriptome profiles impacted by the monoHAAs included genes related to double strand DNA break repair, cell cycle arrest, and apoptosis (programmed cell death) regulation, among which the number of modulated genes involving cell cycle regulation was the highest. It would be interesting to assess if the selected DBPs, monoHAAs and monoHANs would alter the cell cycle progression and if different cell cycle alteration would be observed between these chemicals classes. This is the first study that identified cell cycle alterations by a class of DBPs.

Different from prokaryotic cells, eukaryotic cells have the distinct cell cycle. The cell cycle is divided into interphase (G_1, S, and G_2 phases) and the mitotic phase (M phase) (Figure 1.6 (A)). In the G_1 phase, the cells are preparing for DNA synthesis. S phase is the period of DNA synthesis and G_2 phase is the period when the cells prepare for mitosis. In addition, cells may go into permanent arrest (G_0). Since each chromosome is duplicated during the S phase (2N → 4N; N values denote DNA content as a multiple of the normal haploid genome), cells in G_2 phase have double the amount of DNA (4N) than cells in G_1

phase (2N) (Figure 1.6 (B)). Cell cyclin proteins regulate the cell's progression through the stages of the cell cycle [76]. Activation of checkpoints prevent cells from progressing through the cell cycle for a defined period of time so that there is a longer period available for DNA repair prior to replication, leading to a commensurate reduction in mutation frequency [77].

The variation in the DNA content of cells can be measured using flow cytometry (Figure 1.7). Flow cytometry is a system for sensing cells or particles as they move in a liquid stream through a laser (light amplification by stimulated emission of radiation)/light beam past a sensing area [78]. The fluorochrome propidium iodide binds DNA in a proportional and linear fashion; the intensity of fluorescence is recorded at a single cell level and obtained frequency distribution represents cells with different DNA content, which can represents cell cycle progression [79] (Figures 1.6 (B), 1.7 (A), and 1.8).

1.5 Research Objectives

Even though nearly 40 years have passed since the first discovery of DBPs, the mode of toxic action of DBPs is largely unclear. A comparative and systematic analysis is still lacking. The objective of this research is to develop insights for the mode of toxic action of DBPs by focusing on monohaloacetic acids and monohaloacetonitriles, which are selected as representative carbonaceous and nitrogenous DBPs. Although occurrence and toxicity studies primarily involve dihalo- and trihalo-species, focusing on the simpler monohalogenated species makes it possible to compare the effect of halogen atoms, and the effect of chemical functional group. In this dissertation DNA repair kinetics and cell cycle alteration are used as endpoints for making comparison of these two chemical groups. The objectives of specific chapters are:

- Conduct a DNA repair kinetics study to determine the efficiency of repair of genomic DNA lesions induced by monohaloacetic acids (Chapter 2) (Figure 1.5).
- Conduct a DNA repair kinetics study to determine the efficiency of repair of genomic DNA lesions induced by monohaloacetonitriles, and compare the repair kinetics to that of monohaloacetic acids (Chapter 3) (Figure 1.5).
- Determine the cell cycle alteration induced by monohaloacetonitriles (Chapter 4) (Figure 1.8).

- Determine the cell cycle alteration induced by monohaloacetic acids (Chapter 5) (Figure 1.8).
- Compare cell cycle alteration data by monohaloacetic acids and monohaloacetonitriles.

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Tables and Figures

Table 1.1 Summary of haloacetic acid and haloacetonitriles measured in finished water at drinking water treatment plants (data from [11]).

DBP	Minimum (µg/L)	Median (µg/L)	Maximum (µg/L)
Haloacetic acids			
Monochloroacetic acid	ND	ND	5.5
Monobromoacetic acid	ND	ND	1.6
Dichloroacetic acid	2.2	15	22
Bromochloroacetic acid	1.7	4.4	18
Dibromoacetic acid	ND	2.6	11
Trichloroacetic acid	ND	5.8	20
Bromodichloroacetic acid	ND	3.7	12
Dibromochloroacetic acid	ND	2.1	7.2
Tribromoacetic acid	ND	ND	2.0
Haloacetonitriles			
Chloroacetonitrile	ND	0.05	0.9
Bromoacetonitrile	ND	ND	0.2
Dichloroacetonitrile	ND	0.7	5
Bromochloroacetonitrile	ND	0.8	2
Dibromoacetonitrile	ND	0.5	2
Trichloroacetonitrile	ND	ND	0.1
Bomodichloroacetonitrile	ND	ND	ND
Dibromochloroacetonitrile	ND	ND	ND
Tribromoacetonitrile	ND	ND	ND

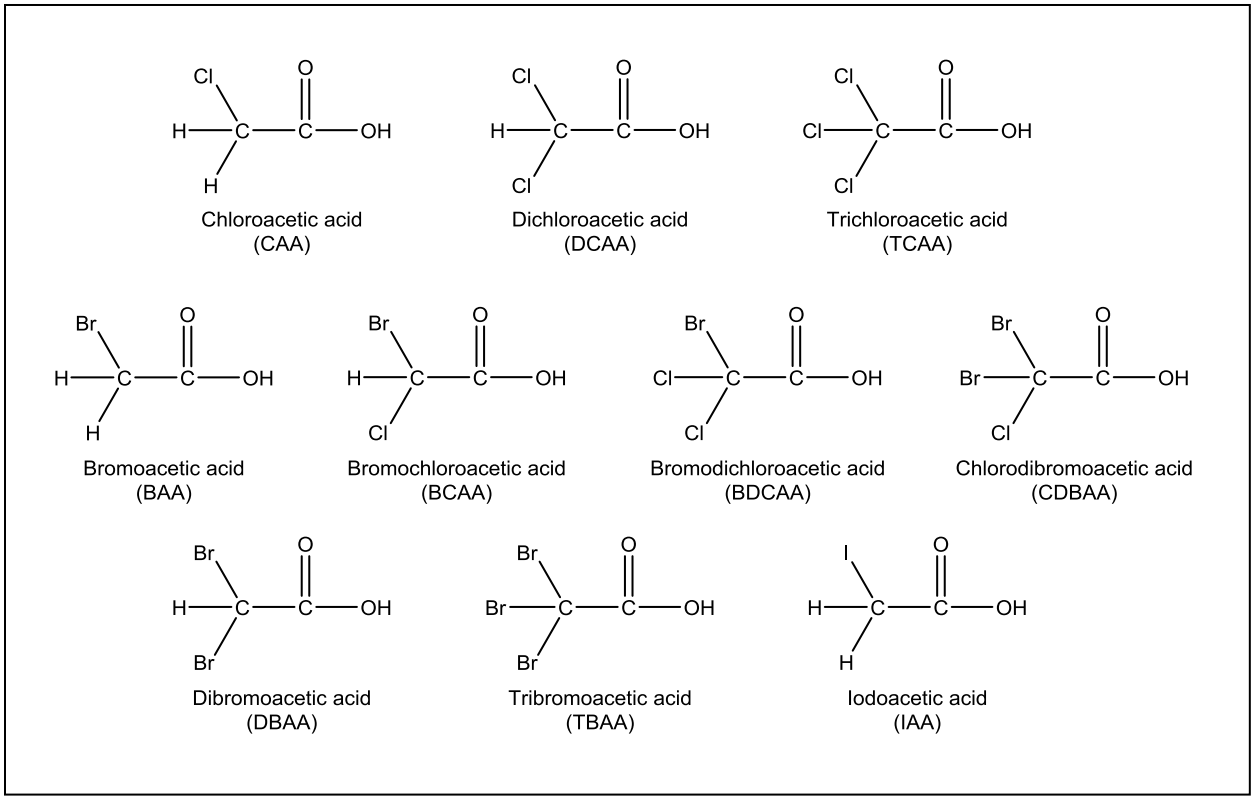

Figure 1.1 Structures of haloacetic acids.

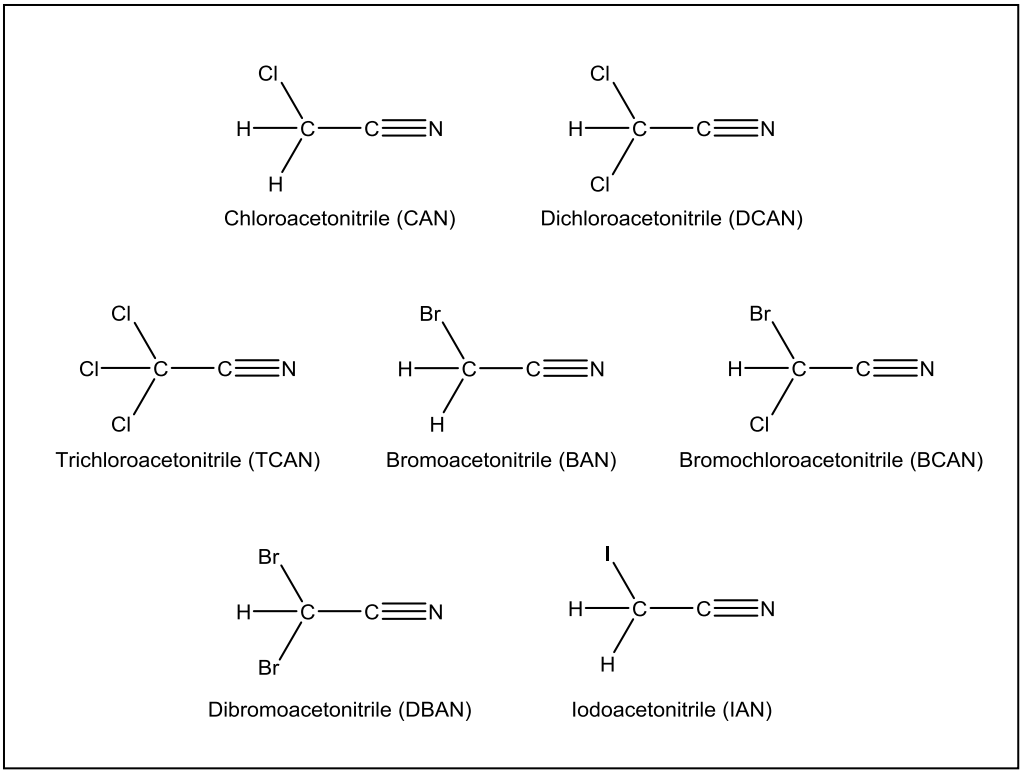

Figure 1.2 Structures of haloacetonitriles.

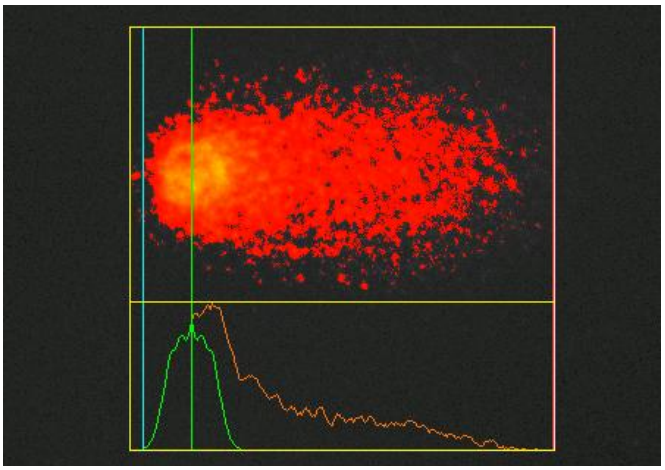

Figure 1.3 Representative image of single cell gel electrophoresis assay illustrating genomic DNA damage in a nucleus. The level of DNA damage is directly related to the amount of DNA that migrates in the microgel.

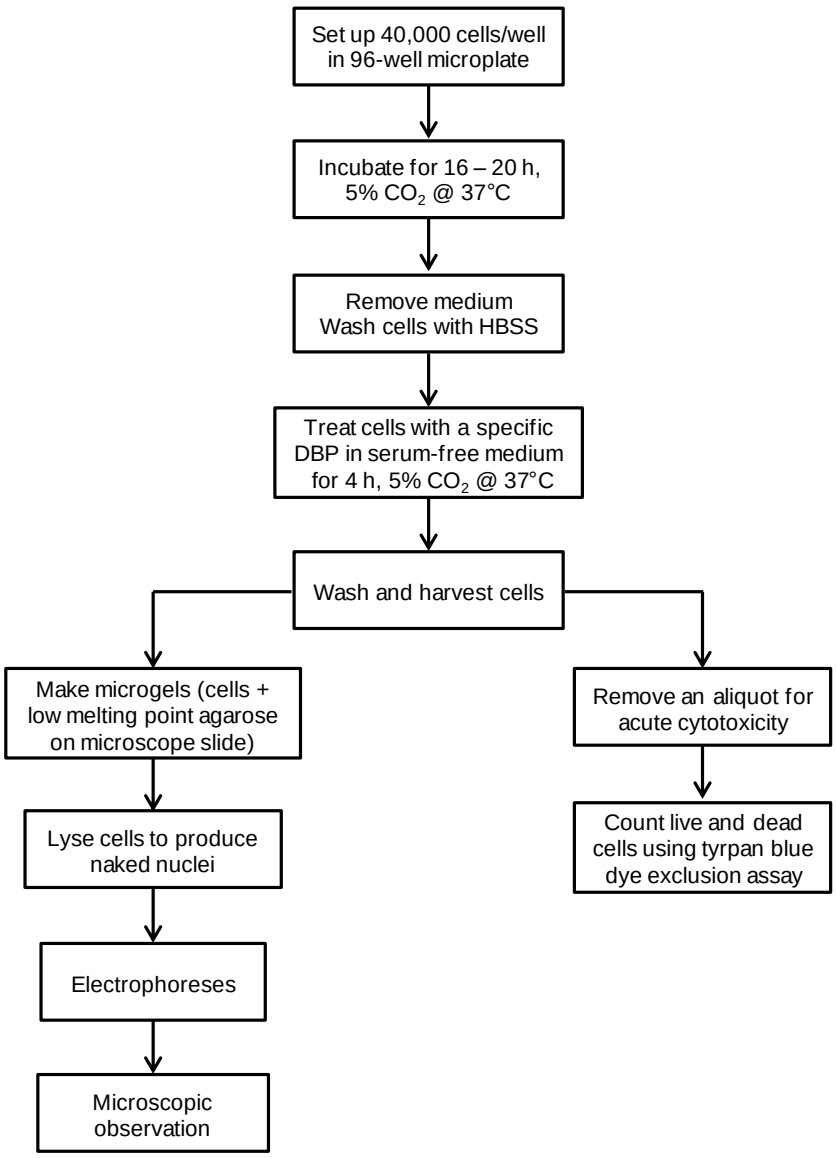

Figure 1.4 Flowchart of the measurement of genomic damage induction using microplate-based single cell gel electrophoresis assay.

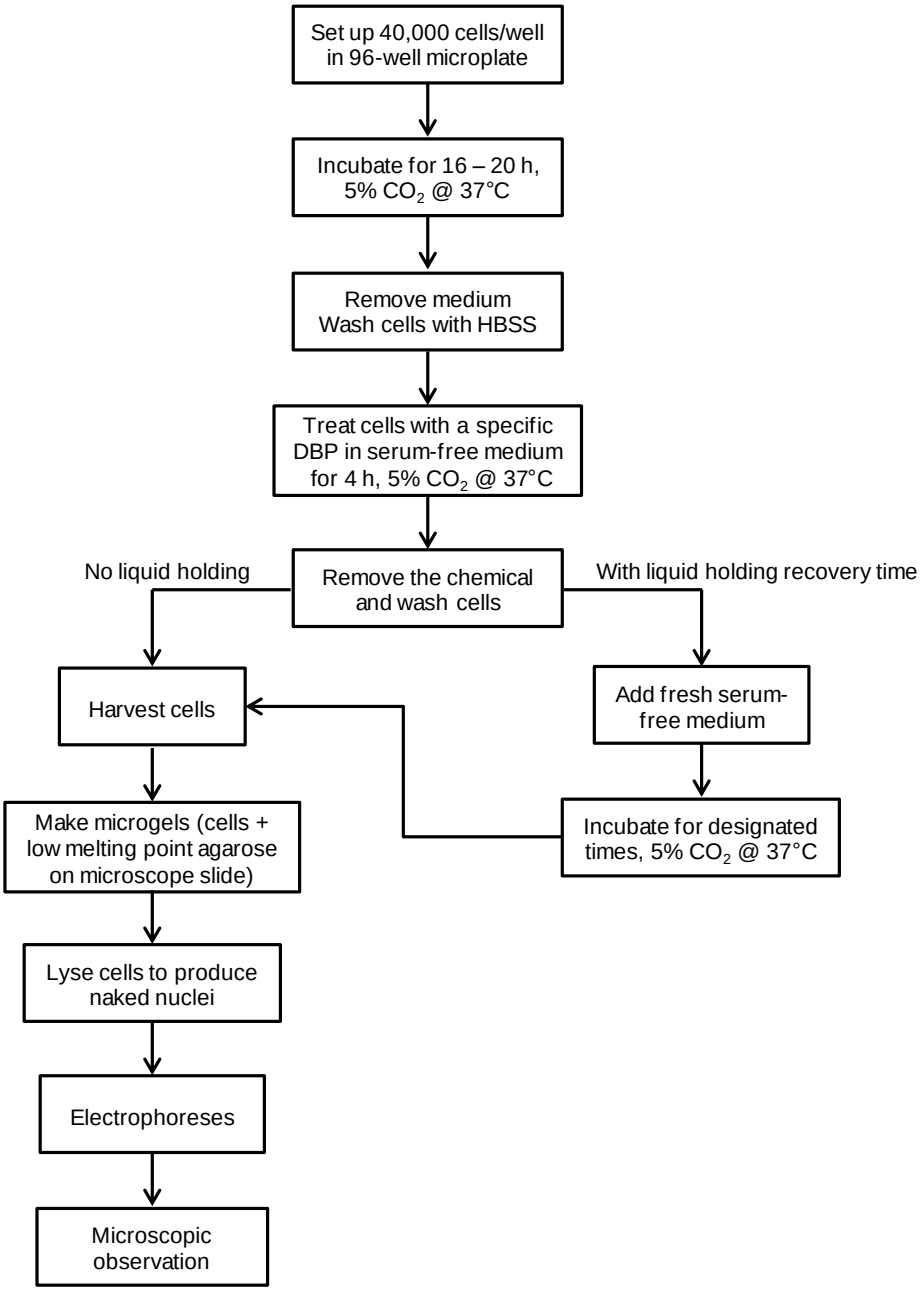

Figure 1.5 Flowchart for measurement of DNA damage repair rate.

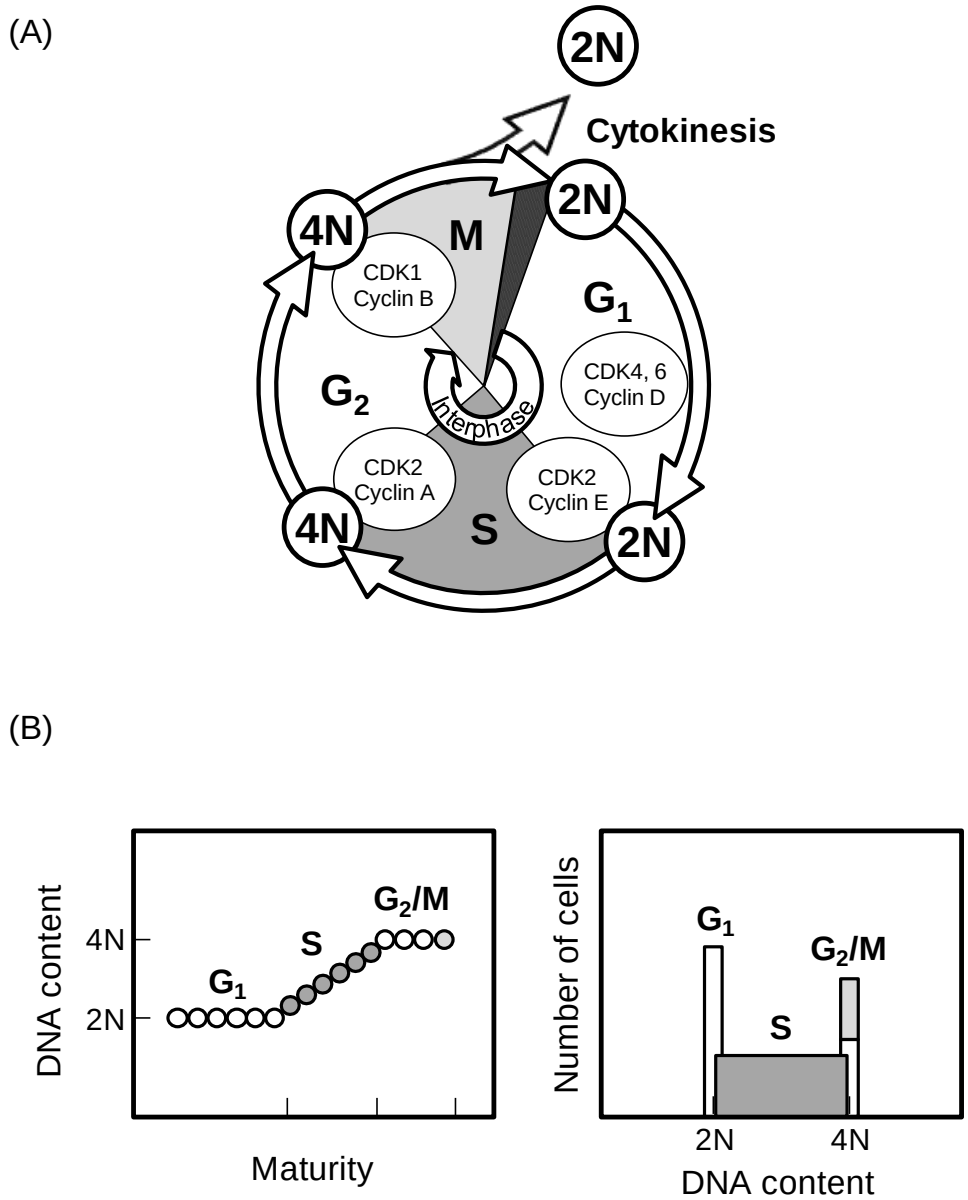

Figure 1.6 (A) Schematic outline of eukaryotic cell cycle and cellular DNA content. (B) Representation of DNA content in each cell cycle.

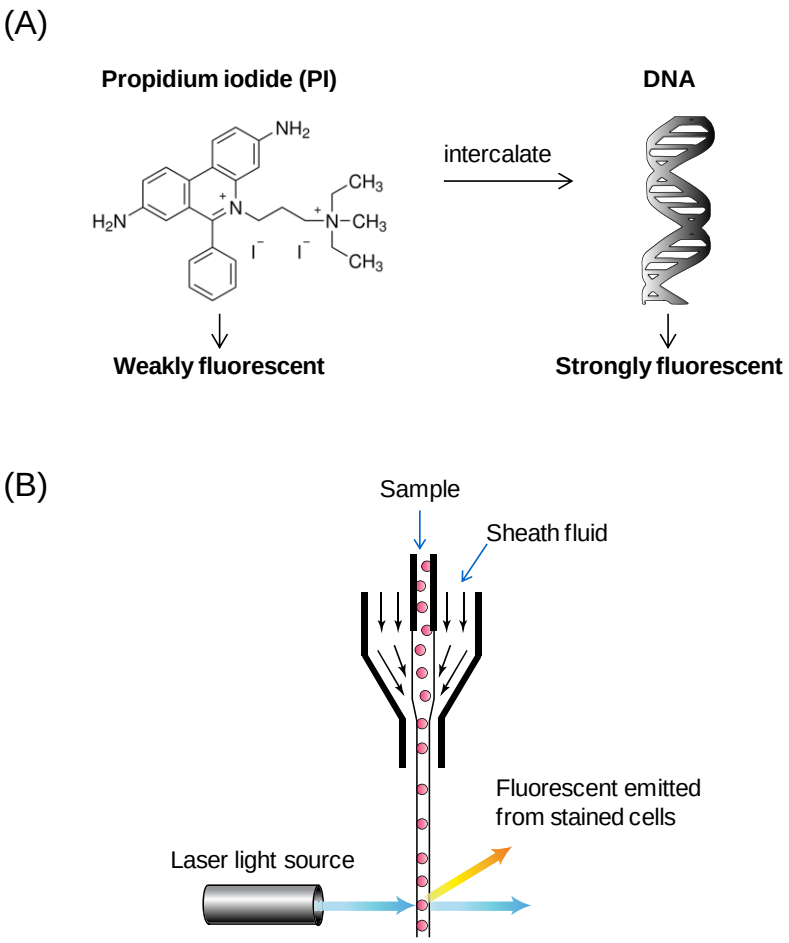

Figure 1.7 (A) Fluorochrome propidium iodide emits strong fluorescence when intercalated into DNA molecules. (B) Schematic diagram of a flow cytometer.

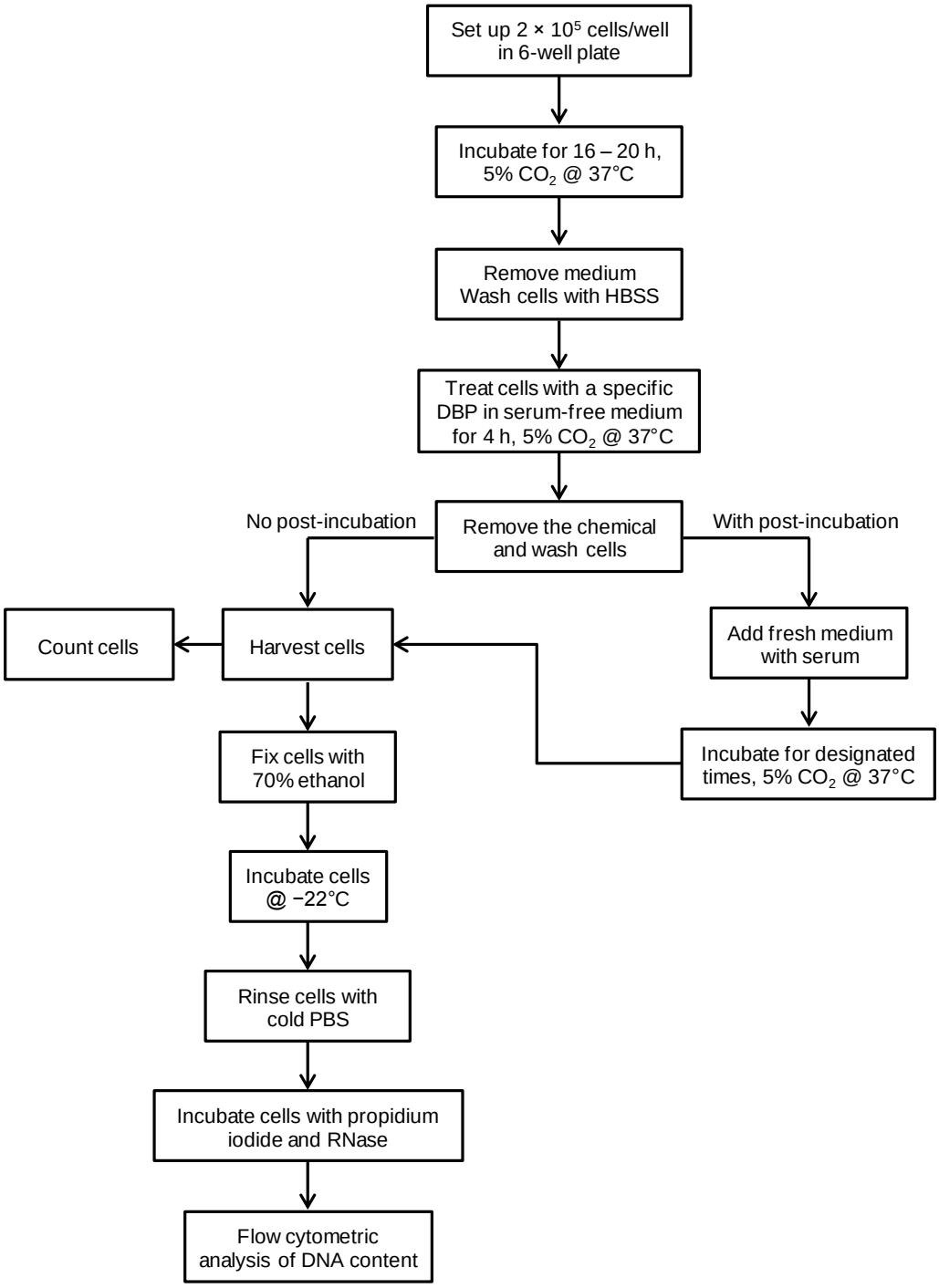

Figure 1.8 Flowchart for cytometric analysis of cellular DNA content.

CHAPTER 2

MAMMALIAN CELL DNA DAMAGE AND REPAIR KINETICS OF MONOHALOACETIC ACID DRINKING WATER DISINFECTION BY-PRODUCTS[1]

ABSTRACT

Haloacetic acids (HAAs) are the second most common class of chlorinated water disinfection by-products (DBPs). The single cell gel electrophoresis genotoxicity assay using Chinese hamster ovary (CHO) cells was modified to include liquid holding recovery time to measure genomic DNA damage and repair kinetics of three monoHAAs: chloroacetic acid (CAA), bromoacetic acid (BAA), and iodoacetic acid (IAA). The rank order of genotoxic potency was IAA > BAA > CAA from previous research. The concentration of each HAA was chosen to generate approximately the same level of genotoxic damage. No cytotoxicity was expressed during the 24-h liquid holding period. Nuclei from CHO cells treated with BAA showed the lowest rate of DNA repair (t_{50} = 296 minutes) compared to CAA or IAA (t_{50} = 134 and 84 minutes, respectively). The different rates of genomic repair expressed by IAA or CAA versus BAA suggest that different distributions of DNA lesions are induced. The use of DNA repair coupled with genomic technologies may lead to the understanding of the biological and genetic mechanisms involved in toxic responses induced by DBPs.

2.1 Introduction

The drinking water community provides an exceedingly important public health service by generating high quality, safe and palatable tap water. Each day approximately 250,000 public water purification facilities in the United States provide drinking water to 90% of the population [1]. During the twentieth century the outbreak of waterborne diseases including typhoid and cholera were greatly suppressed

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[1] Reprinted with permission from Komaki, Y.; Pals, J.; Wagner, E. D.; Mariñas, B. J.; Plewa, M. J., Mammalian cell DNA damage and repair kinetics of monohaloacetic acid drinking water disinfection by-products. *Environ. Sci. Technol.* **2009**, 43, (21), 8437-42. Copyright 2009 American Chemical Society.

through disinfection [2]. However, disinfectants react with natural constituents, e.g., organic matter, bromide and iodide, in source water and form disinfection by-products (DBPs); this unintended consequence may cause negative health effects [3]. In order to reduce exposure, the U.S. Environmental Protection Agency (U.S. EPA) promulgated the Stage 1 Disinfectants and Disinfection Byproducts Rule (DBPR) in 1998 [4] and the Stage 2 DBPR in 2006 [5]. In chlorinated water, haloacetic acids (HAAs) are the second most common class of DBPs [6]. The sum of five HAAs (monochloro-, dichloro-, trichloro-, monobromo- and dibromoacetic acid) is regulated at a maximum contaminant level of 60 μg/L [4, 5].

The occurrence, genotoxicity and carcinogenicity of the HAAs were recently reviewed [7]. All five regulated HAAs were mutagenic in bacteria [8] and induced genomic DNA damage in mammalian cells *in vitro*. Among the three monohaloacetic acids, iodoacetic acid (IAA) was the most cytotoxic and genotoxic followed by bromoacetic acid (BAA) and chloroacetic acid (CAA) [9, 10]. The rank order of their toxicity correlated with their electrophilic reactivity and the carbon-halogen bond dissociation energy [9]. The rank order of teratogenic potency (neural tube developmental defects in mouse embryos) was IAA > BAA > CAA [11, 12]. In general, brominated acetic acids were consistently more cytotoxic and more genotoxic than the corresponding chlorine substituted acids [7].

Although there are many studies on the induction of DNA damage by DBPs, little information exists on the repair of DBP-induced DNA lesions [13, 14]. In the literature there is no systematic analysis of the DNA repair kinetics of regulated DBPs nor is there an example of correlating chemical structure activity relationships and repair. The aim of this research was to characterize the genotoxicity induced by monoHAAs in mammalian cells and determine the kinetics of DNA repair. We employed HAA concentrations that induced approximately equivalent genotoxic effects. Our hypothesis was that if DNA lesions induced by these HAAs were similar then no statistical difference would be observed in the kinetics of DNA repair. If the different halogens (I, Br, Cl) induced different DNA lesions then the DNA repair kinetics would be significantly different.

2.2 Materials and Methods

2.2.1 Reagents

General reagents were purchased from Fisher Scientific Co. (Itasca, IL) and Sigma Chemical Co. (St. Louis, MO). Bromoacetic (BAA) and chloroacetic acid (CAA) were purchased from Fluka Chemical Co. (Buchs, Switzerland). Iodoacetic acid (IAA) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Cell culture F12 medium and fetal bovine serum (FBS) were purchased from Fisher Scientific Co. The HAAs were dissolved in dimethyl sulfoxide (DMSO) and stored at −22°C in sealed, sterile glass vials.

2.2.2 Chinese hamster ovary cells

Clone 11-4-8 of the transgenic Chinese hamster ovary (CHO) cell line AS52 was used [15]. The cells were maintained in F12 medium containing 5% FBS, 1% antibiotic-antimycotic solution (Invitrogen, Carlsbad, CA) and 1% glutamine, and grown in 100 mm glass culture plates at 37°C in a humidified atmosphere of 5% CO_2.

2.2.3 Treatment conditions

The day before treatment, 4×10^4 CHO cells were plated in 200 μL of F12 + 5% FBS medium per well in a sterile flat-bottom 96-well microplate. The next day, the cells were washed with Hank's balanced salt solution (HBSS) and treated with the HAAs in F12 medium without FBS in a total volume of 25 μL for 4 hours at 37°C, 5% CO_2. The wells were covered with AlumnaSeal™. The concentrations of the HAAs for the DNA repair studies were first determined so that similar levels of genomic DNA damage were induced. The concentrations were 6 mM, 60 μM, and 25 μM for CAA, BAA and IAA, respectively. After treatment, the solution was aspirated and the cells were washed 2× with HBSS. The cells from one well were immediately harvested and microgels were prepared for the determination of DNA damage with no time for repair. F12 medium without FBS (100 μL) was added to the other wells, and the microplate was returned to the incubator for designated times. This recovery period (liquid holding time) allowed for DNA repair.

2.2.4 Single cell gel electrophoresis

Single cell gel electrophoresis (SCGE, or the Comet assay) quantitatively measures genomic DNA damage induced in individual nuclei of treated cells [16, 17] and has been used as a predictor of carcinogenic activity [18]. The Comet assay is rapid and sensitive, and detects lesions including DNA single-strand and double-strand breaks, incomplete excision repair sites, and alkali-labile sites. The assay combined with liquid holding allows for a quantitative analysis of DNA repair [13, 14, 17, 19, 20].

Detailed methods for preparation of SCGE slides and electrophoresis have been published previously [9, 10]. After treatment the cells were detached with 50 μL of a 0.005% trypsin solution in HBSS, followed by the addition of 70 μL F12 + 5% FBS. An aliquot was analyzed for acute cytotoxicity with the trypan blue dye exclusion assay [21]. The remaining cell suspension was embedded in a layer of low melting point agarose prepared in phosphate-buffered saline on clear microscope slides that were pre-coated with 1% solution of normal melting point agarose prepared with deionized water. Two SCGE microgels were prepared from the suspension harvested from each well. The microgels were placed in lysing solution (2.5 M NaCl, 1% sodium lauryl sarcosinate, 100 mM Na_2EDTA, 10 mM Tris, pH 10 with 1% Triton X-100 and 10% DMSO) overnight at 4°C.

The DNA of the nuclei was denatured for 20 minutes in electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH 13.5). The SCGE microgels were electrophoresed for 40 minutes at 25 V and 300 mA (0.72 V/cm) at 4°C. After neutralization with 400 mM Tris buffer (pH 7.5) the microgels were dehydrated in cold methanol for 20 minutes, dried for 5 minutes at 50°C, and stored at room temperature. For analysis, the microgels were hydrated in water at 4°C for 20 minutes, and then the nuclei were stained with 65 μL of an ethidium bromide solution (20 μg/mL) and rinsed in cold water. The microgels were kept at 4°C and analyzed within a 4-hour time period.

Fifty randomly chosen nuclei per microgel were analyzed with a Zeiss fluorescence microscope (excitation filter of BP 546/10 nm, barrier filter of 590 nm) and a computerized image analysis system

(Komet version 3.1, Kinetic Imaging Ltd., Liverpool, UK). The digitalized data were automatically transferred from the CCD camera to a computer-based spreadsheet for statistical analysis.

2.2.5 Statistical analysis

Each experiment was independently repeated three times. The data were transferred to Excel spreadsheets (Microsoft Corp., Redmond, WA) and analyzed using SigmaStat 3.1, SigmaPlot 8.0 programs and TableCurve 2D (SPSS Inc. Chicago, IL). The primary measure of DNA damage and repair was the percent of the DNA that had migrated from the nucleus into the microgel (% tail DNA).

The distributions of the % tail DNA values are not normal. In order to conduct parametric statistical analysis the mean % tail DNA value was calculated for each microgel. The mean % tail DNA values for each experimental group were averaged. According to the central limit theorem, these averaged mean values were normally distributed [22]; the data were analyzed using a two-way analysis of variance test statistic. The mean % tail value from the HAA-treated cells with no recovery time was set at 100% and compared with concurrently treated cells after designated periods of liquid holding. If a significant F value of $P \leq 0.05$ was obtained, a Holm-Sidak multiple-comparison was conducted in order to determine if there was a significant difference in the rate of DNA repair among the monoHAAs.

2.3 Results and Discussion

All three monoHAAs induced genomic DNA damage. In this study the concentration of each HAA was selected to induce similar levels of genotoxic damage. The % tail DNA for each HAA at 0-time liquid holding was normalized to a value of 100% that allowed for a direct comparison of the rates of DNA repair.

2.3.1 Liquid holding time and cytotoxicity

CHO cell viability was determined throughout a 60-hour time period under liquid holding conditions (F12 medium without FBS). More than 55 hours of liquid holding time caused a decline in viability

(Figure 2.1). There was no decrease in viability during the 24-hour liquid holding period that was used for the DNA repair experiments.

2.3.2 DNA repair kinetics

DNA repair kinetics was determined for IAA, BAA, and CAA using SCGE and a liquid-holding protocol [16, 17] (lower plot, Figure 2.2). The data for each DNA repair curve were regressed using curvilinear curve fitting as well as regression based on first order kinetics (Table 2.1). The time required to affect 50% repair of the originally induced DNA damage (t_{50}) was calculated by sigmoidal or first order regression analysis within the 0 – 24 hour liquid holding time. With sigmoidal regression, the t_{50} values for IAA, CAA and BAA were 84, 134 and 296 minutes, respectively (Table 2.1). The values were similar with first order regression. Using first order regression analysis, bimodal repair kinetics was observed. These bimodal repair curves may be due to the diversity of DNA lesions induced by the HAAs. Apurinic sites, single strand DNA breaks, and damaged bases can be repaired more efficiently and rapidly as compared to DNA crosslinks and DNA double strand breaks. These latter lesions induce serious genomic damage and require more cellular processing for repair [23]. The calculated first order repair rate (during the first ~3 hours of liquid holding) for IAA, CAA and BAA was 0.842 $hour^{-1}$, 0.763 $hour^{-1}$ and 0.187 $hour^{-1}$, respectively. The rate of repair of BAA-induced lesions was different from the repair rates of CAA or IAA. Acute cytotoxicity was not observed throughout the liquid holding period (upper plot, Figure 2.2).

2.3.3 Repair of DNA damage induced by the monohaloacetic acids

Cells treated with BAA expressed a lower rate of DNA repair compared to IAA or CAA (Figure 2.2). To determine if there were significant differences in DNA repair at each liquid holding time we used a balanced multivariant analysis of variance (ANOVA) with a Holm-Sidak pairwise multiple comparison test (Table 2.2). A significant difference in DNA repair kinetics was expressed by CHO cells exposed to BAA as compared to IAA or CAA. The DNA repair kinetics for CAA and IAA were statistically similar.

2.3.4 Frequency distribution of repaired nuclei

The levels of % tail DNA for each monoHAA as a function of liquid holding time were analyzed with frequency distribution histograms. Figure 2.3 illustrates the dynamics of genomic DNA repair in which the nucleus was the unit of measure. For each nucleus the distribution of DNA migration during each liquid holding time was plotted. The concentrations of monoHAAs were chosen to generate approximately similar levels of biological damage. The 0-hour liquid holding time (Figure 2.3, far left panel of stacked plots) shows that CAA and IAA induced similar profiles of DNA damage. The damage induced by BAA with no liquid holding was much broader with fewer nuclei with the highest % tail DNA values but also many nuclei with lower amounts of damage. As the liquid holding time progressed from 3 to 24 hours, the levels of DNA damage in each treatment group diminished shifting the distributions to the left in each panel. However, the population of nuclei damaged by BAA showed lesser repair as compared to CAA and IAA (two center vertical panels). Few unrepaired nuclei remained after 24-hour liquid holding (Figure 2.3, far right panel). DNA damage histograms for the untreated negative controls are presented in Figure 2.4; over a 24-hour time period no significant difference was observed in DNA damage distributions. The level of completeness for the repair of DNA damage induced by each monoHAA can be observed by comparing the 24-hour liquid holding distribution (Figure 2.3, far right panel) with that of the negative control (Figure 2.4).

With no liquid holding time for repair, the mean of the % tail DNA was 60.5, 50.3, and 67.3 for IAA, BAA and CAA, respectively. After 24 hours of liquid holding the mean % tail DNA values were 7.5, 12.0 and 7.2, respectively. Liquid-holding recovery significantly shifted the distributions to lower % tail DNA values; IAA showed the most rapid shift, CAA the next and BAA the slowest (Figure 2.3). The mean % tail DNA values of the negative control did not change significantly: 4.8% without liquid holding, 4.1% after 5 hours and 4.3% after 24 hours liquid holding recovery time (Figure 2.4).

The different rates of genomic repair that are expressed by IAA or CAA versus BAA suggest that different DNA lesions and/or different distributions of DNA lesions are induced. Since the concentration of each haloacetic acid was chosen to generate approximately the same level of genotoxic damage, the

null hypothesis was that there would be no difference in the DNA repair kinetics if similar DNA lesions were induced by the three haloacetic acids. The data do not support the null hypothesis. Currently there is not a complete analysis of the types of DNA lesions induced by the haloacetic acids.

Cemeli et al. showed that the antioxidant butylated hydroxyanisole or catalase substantially reduced IAA-induced mutagenicity in *Salmonella typhimurium* and reduced genomic DNA damage in CHO cells [24]. Oxidative stress may be a biological mechanism contributing to the genotoxicity of IAA. Oxidative stress has been linked with the toxicity of other DBPs including 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX) [25], bromate [26-29], and chloroacetonitrile [30]. Oxidative stress-induced DNA damage ranges from individual base damage to single strand and double strand DNA breaks [23]. DNA repair of oxidative stress-induced lesions may be relatively fast if mediated by DNA glycosylases [31] or require more time if the lesion is a double strand DNA break [32].

Recent toxicogenomic data indicate that non-cytotoxic concentrations of BAA preferentially modulate the expression of human genes involved in double strand DNA (dsDNA) break repair [33]. Double strand breaks are one of the most toxic and mutagenic lesions and require more time for repair than other types of DNA damage [32]. dsDNA breaks must be repaired to restore the integrity, stability and reproducibility of the genome. *XRCC3-241* is a mutant allele for dsDNA break repair; humans who carry this allele express higher risks of bladder cancer [34]. It is intriguing that bladder cancer is associated with the consumption of disinfected drinking water [35].

The use of DNA repair coupled with genomic technologies may lead to the understanding of the biological and genetic mechanisms that are involved in toxic responses induced by DBPs. Such knowledge may lead to the identification of biomarkers that may be employed in feed-back loops to aid water chemists and engineers in the overall goal of producing safer drinking water.

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Tables and Figures

Table 2.1 DNA repair t_{50} values of genomic damage induced by IAA, BAA or CAA.

Haloacetic Acid	Treatment Concentration	CAS Number	t_{50} (min, sigmoidal regression)	t_{50} (min, first order regression)
IAA	25 µM	64-69-7	84	85
BAA	60 µM	79-08-3	296	319
CAA	6 mM	79-11-8	134	135

Table 2.2 Two-way Analysis of Variance for DNA repair of CHO cells treated with IAA, BAA or CAA (Holm-Sidak pairwise multiple comparison).

Comparison	Liquid Holding Time (h)	Difference of Means	*P*
CAA vs. IAA	0	1.42×10^{-13}	0.99
CAA vs. BAA	0	4.26×10^{-14}	0.99
BAA vs. IAA	0	9.95×10^{-14}	0.99
CAA vs. IAA	1	4.08	0.58
CAA vs. BAA *	1	25.22	0.001
BAA vs. IAA *	1	21.14	0.005
CAA vs. IAA *	2	15.70	0.03
CAA vs. BAA *	2	19.35	0.009
BAA vs. IAA *	2	35.05	0.0001
CAA vs. IAA	3	12.79	0.08
CAA vs. BAA *	3	23.35	0.002
BAA vs. IAA *	3	36.136	0.0001
CAA vs. IAA	4	11.46	0.1
CAA vs. BAA *	4	19.90	0.007
BAA vs. IAA *	4	31.36	0.0001
CAA vs. IAA	5	7.49	0.3
CAA vs. BAA	5	13.87	0.06
BAA vs. IAA *	5	21.36	0.004

* DNA repair levels were statistically different between the treatment groups at $P < 0.05$.

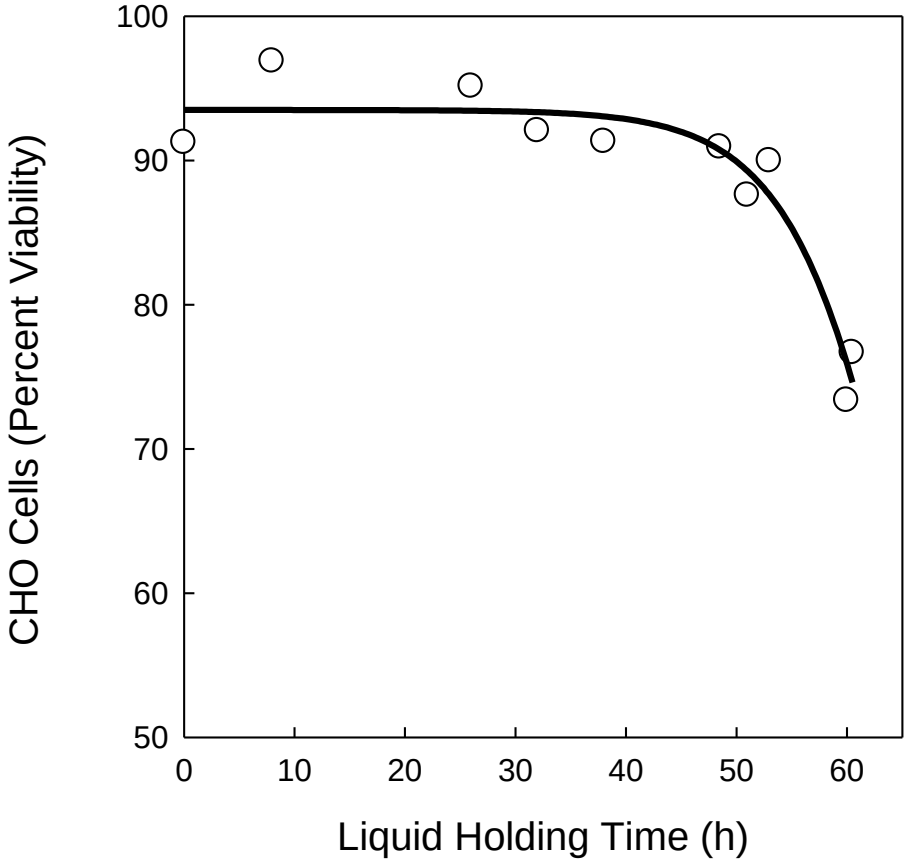

Figure 2.1 CHO cell viability throughout a 60-hour time period with cells maintained under liquid holding conditions (F12 medium without FBS).

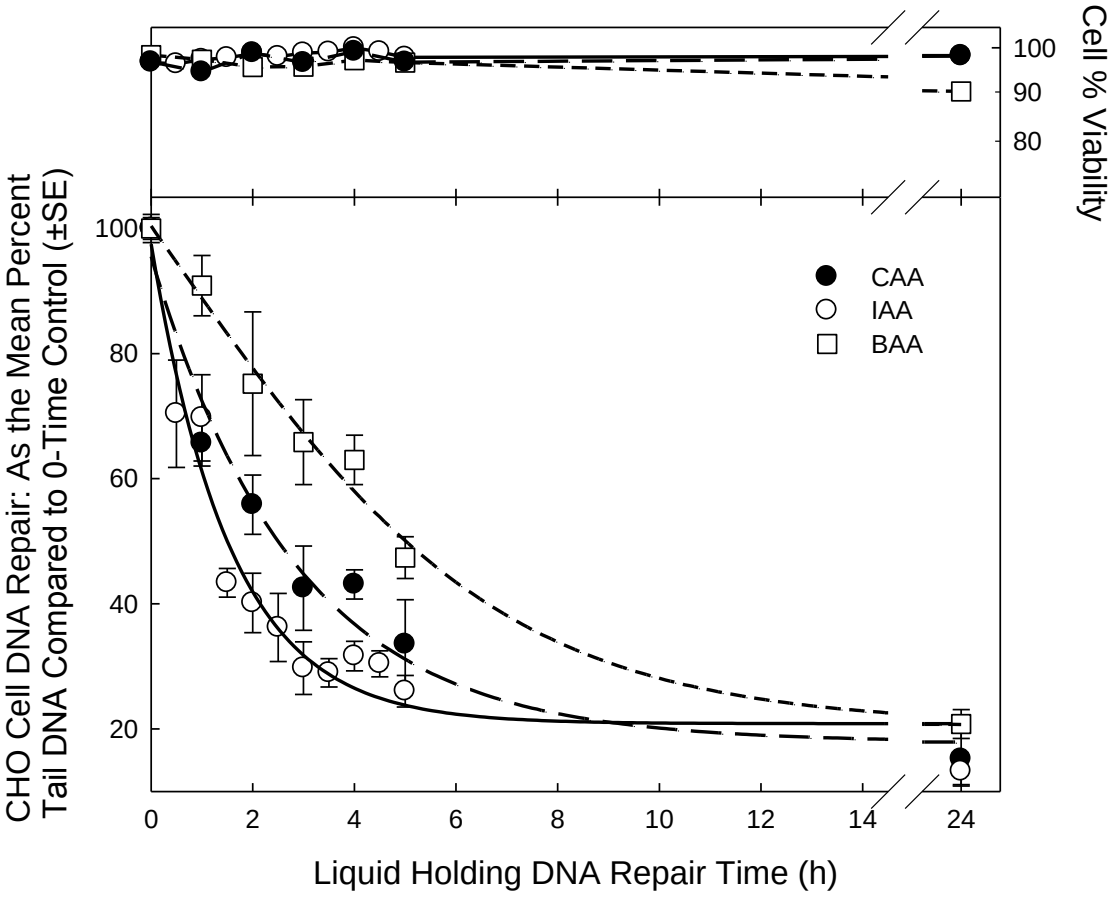

Figure 2.2 Comparative genomic DNA repair kinetics in CHO cells for chloroacetic acid (CAA), iodoacetic acid (IAA), and bromoacetic acid (BAA).

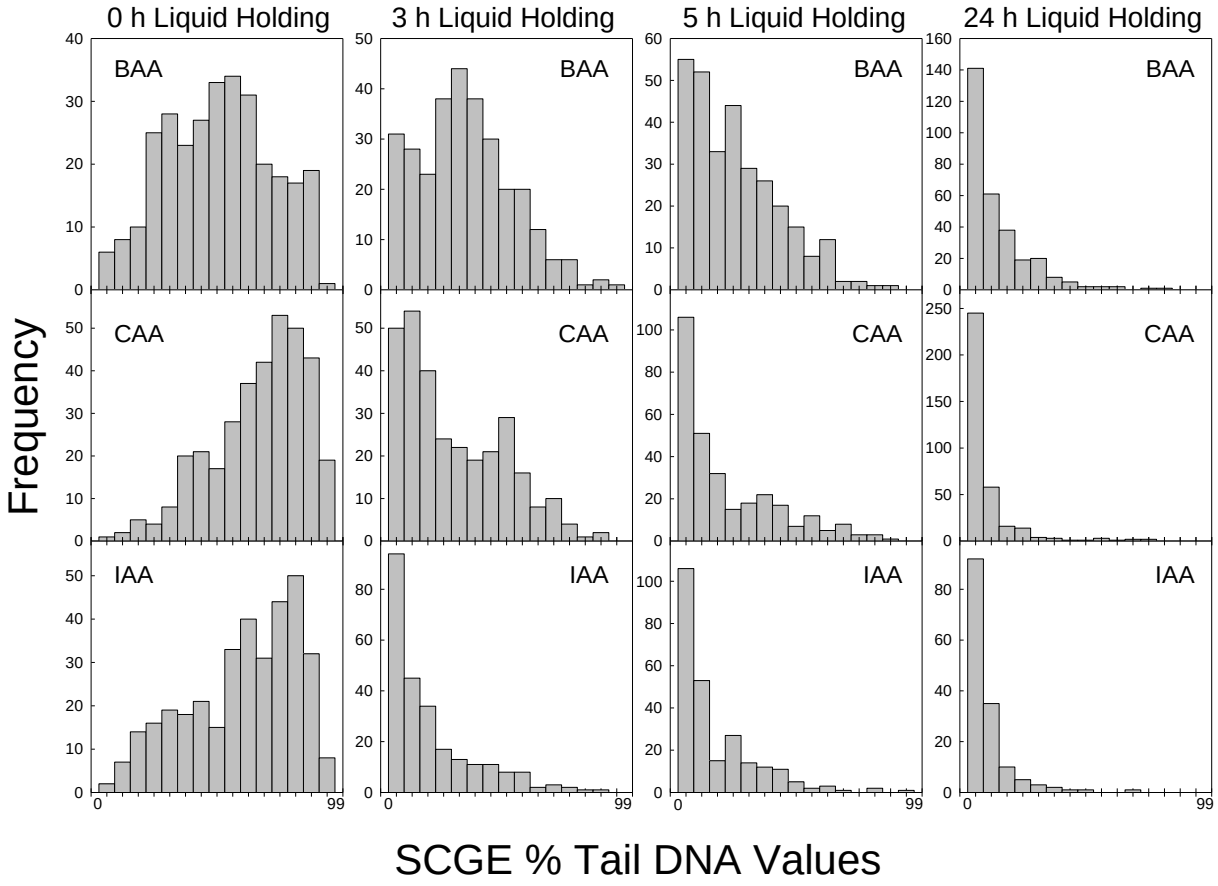

Figure 2.3 Histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with bromoacetic acid (BAA), chloroacetic acid (CAA) and iodoacetic acid (IAA) without and with 3, 5 and 24 hour liquid-holding recovery.

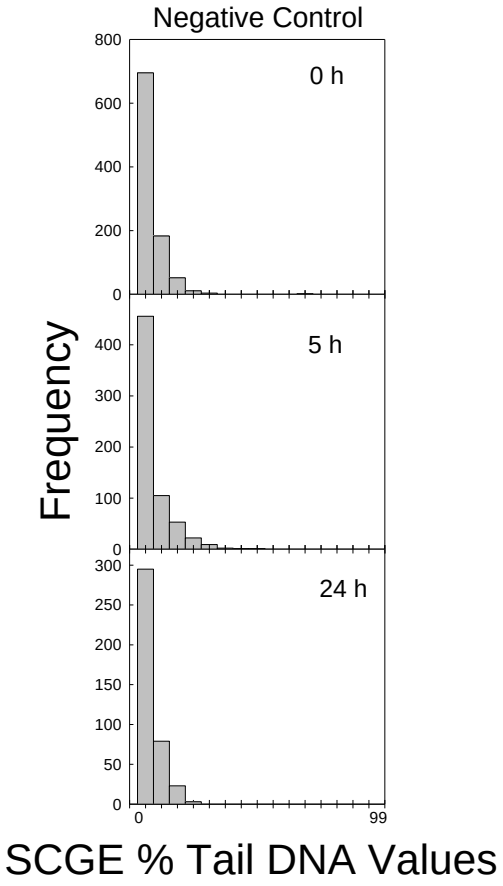

Figure 2.4 Histograms illustrating the distributions of SCGE % tail DNA values from the CHO cell negative controls at 0, 5 and 24 hour liquid-holding recovery. No significant difference was observed among the three negative control groups ($F_{2, 1997} = 2.81$; $P = 0.06$).

CHAPTER 3

DNA REPAIR KINETICS OF THREE MONOHALOACETONITRILES

ABSTRACT

Haloacetonitriles (HANs) are a chemical class of DBPs that form from reactions between disinfectants and organic nitrogen agents present in source waters. Increasing use of source waters impacted by wastewater effluents, algal blooms, or anthropogenic contaminants may enhance the formation of HANs. HANs are cytotoxic, mutagenic, genotoxic and developmentally toxic. DNA damage induction, DNA damage repair and cell cycle regulation are strongly associated. Measuring the efficiency of DNA damage repair should provide an insight on HAN-induced lesions and the impact of different halogen atom substitution.

The single cell gel electrophoresis (SCGE) assay was used to 1) determine the induction of single and double strand DNA breaks and 2) determine the DNA repair kinetics of HAN-induced genetic damage. In addition, the plating efficiency of HAN-treated cells was evaluated. The SCGE assay demonstrated that the monoHANs were genotoxic, with the descending rank order of IAN > BAN > CAN. Induced genomic DNA damage was repaired with very similar rates among the HANs. The contribution of the halogen atoms in each HAN on DNA repair kinetics appeared to be different from previously measured HAAs, suggesting differences in the modes of toxic action between these two chemical groups.

3.1 Introduction

Disinfection processes provide a primary defense against waterborne infectious disease [1] yet in addition to inactivating pathogens disinfectants can also react with precursors, mainly natural organic matter, in source waters to form disinfection byproducts (DBPs). Many DBPs are genotoxic, carcinogenic and teratogenic [2, 3]. Epidemiological studies have linked chlorinated water consumption with cancer and adverse reproductive outcomes [4]. Recent *in vitro* mammalian cell toxicity assays demonstrated that haloacetonitriles (HANs), haloacetamides, and halonitromethanes were significantly more geno- and

cytotoxic than regulated trihalomethanes (THMs) and haloacetic acids (HAAs) [5-7] and this raised interests toward investigating nitrogenous disinfection byproducts (N-DBPs) [8]. Moreover, the increasing use of source waters impacted by municipal wastewater effluent discharge or algal blooms may promote N-DBP formation due to elevated levels of organic nitrogen content [9-14]. HANs are a chemical class of DBPs first identified in the 1980s [15, 16] and were known to form from the chlorination of amino acids [17] as well as during chloramination [18]. HANs are widely detected in water treatment plants and disinfected wastewater effluents [19, 20] as well as in swimming pool water [21, 22]. Besides the ingestion route, HANs can be absorbed via percutaneous absorption [23, 24].

The metabolism and toxicity of HANs were reviewed previously [25]. Toxicological studies of HANs primarily involved chlorinated and brominated HANs. Chloroacetonitrile (CAN), dichloacetonitrile (DCAN), trichloroacetonitrile (TCAN) and bromochloroacetonitrile (BCAN) were mutagenic in *Salmonella typhimurium* [26-28]. HANs bound to DNA and produced DNA strand breaks in human cultured lymphoblastic cells; however, there was no correlation between these two endpoints [29]. CAN and DCAN can cross the placental barrier and accumulate in the fetal brain [30-32]. Dibromoacetonitrile (DBAN) was found to be carcinogenic in rats and mice [3, 33] and the contribution of this HAN to lifetime risk of cancer was calculated to be higher than that of THMs using a quantitative structural toxicity relationship model [34]. A systematic toxicology study including iodinated HAN was not published until 2007; *in vitro* mammalian cell toxicity demonstrated a descending rank order of DBAN > bromoacetonitrile (BAN) ≈ iodoacetonitrile (IAN) > BCAN > DCAN > CAN > TCAN for chronic cytotoxicity, and IAN > BAN ≈ DBAN > BCAN > CAN > TCAN > DCAN for acute genotoxicity [7].

The objective of this study was to enhance our knowledge of the genotoxicity of HANs using Chinese hamster ovary (CHO) cells. The single cell gel electrophoresis (SCGE) assay was used to 1) determine the induction of single and double strand DNA breaks and 2) determine the DNA repair kinetics of the induced genetic damage. The SCGE assay is a sensitive tool for quantifying genomic DNA damage and repair [35-39]. SCGE was also used as a predictor of carcinogenic activity [40]. SCGE is widely used for applications including human biomonitoring, genotoxicity, ecological monitoring and as a tool to

investigate DNA damage and repair in different cell types in response to a wide range of DNA-damaging agents [37, 41]. SCGE assay allows for repair kinetics experiments by measuring the remaining unrepaired DNA as a function of time [42-47]. This approach determines the efficiency of the repair process toward different DNA lesions. Biological systems have DNA repair capability for DNA damage to maintain genomic integrity and stability. Basic DNA repair mechanisms include direct DNA repair, base excision repair, nucleotide excision repair, mismatch repair, homologous recombination, and non-homologous end joining [48]. DNA repair kinetic experiments may provide insights on HAN-induced lesions and if they persist as a result of different halogen atom substitution. Moreover, a comparison with previously conducted HAA repair kinetics may provide insights on the contribution of halogen atoms among different chemical groups, such as HANs and HAAs. In this work, DNA repair kinetics and the plating efficiency of HAN- and HAA-treated cells were investigated. It is commonly believed that eukaryotic cells delay the cell cycle to allow DNA repair to proceed. A damage recognition sensor triggers a signal transduction cascade and downstream effectors toward cell cycle arrest [49-53]. The checkpoint response is also associated with mutagenic translesion DNA synthesis [54]. We therefore hypothesized that faster translesion repair might be associated with induced cell cycle arrest.

3.2 Materials and Methods

3.2.1 Reagents

General reagents were purchased from Fisher Scientific Co. (Itasca, IL) and Sigma-Aldrich Co. (St. Louis, MO). Iodoacetonitrile (98%, CAS# 624-75-9), bromoacetonitrile (97%, CAS# 590-17-0) and chloroacetonitrile (99%, CAS# 107-14-2) were purchased from Sigma-Aldrich. Bromoacetic acid (≥ 99%, CAS# 79-08-3) and chloroacetic acid (≥ 99%, CAS# 79-11-8) were purchased from Fluka Chemical Co. (Buchs, Switzerland). Iodoacetic acid (≥ 99%, CAS# 64-69-7) was purchased from Aldrich Chemical Co. (Milwaukee, WI). HANs and HAAs were diluted in dimethyl sulfoxide (DMSO) at 1 mol/L and stored at −22°C.

3.2.2 Chinese hamster ovary cells and culture media

Transgenic Chinese hamster ovary (CHO) cells line AS52 clone 11-4-8 [55, 56] were maintained in modified Ham's F12 medium (Mediatech, Inc., Manassas, VA) supplemented with 5% fetal bovine serum (FBS), 1% L-glutamine and 1% antibiotic-antimycotic solution (10 units/mL penicillin G sodium, 10 μg/mL streptomycin sulfate, 25 μg/mL amphotericin B, 0.85% saline; Invitrogen, Carlsbad, CA). The cells exhibit normal morphology, express cell contact inhibition and grow as a monolayer without expressing neoplastic foci. The cells were grown in 100-mm glass culture plates at 37°C in a humidified atmosphere containing 5% CO_2.

3.2.3 Treatment conditions

The day before treatment, 4×10^4 CHO cells were plated in 200 μL of F12+5% FBS per well in a sterile flat-bottom 96-well microplate. The next day, the cells were washed with divalent cation-free Hank's balanced salt solution (HBSS) and treated with the HANs in serum-free F12 medium in a total volume of 25 μL for 4 hours at 37°C, 5% CO_2. The wells were covered with AlumnaSeal™ to prevent chemical volatilization. After treatment, the solution was aspirated and the cells were washed twice with HBSS. The cells from one well were immediately harvested and microgels were prepared for the determination of DNA damage with no time for repair. Fresh serum-free F12 medium (100 μL) was added to the other wells, and the microplate was returned to the incubator for designated times. This recovery period (liquid holding time) allowed for cells to repair their induced lesions.

3.2.4 Single cell gel electrophoresis

The SCGE assay is rapid, sensitive, and detects lesions including DNA single-strand and double-strand breaks, incomplete excision repair sites, and alkali-labile sites. The assay combined with liquid holding allows for a quantitative analysis of DNA repair [39, 42-47]. Detailed methods for preparation of SCGE slides and electrophoresis have been published previously [57-59]. After treatment the cells were detached with 50 μL of a 0.025% trypsin + 0.01% EDTA solution in HBSS, followed by the addition of

70 μL F12 + 5% FBS. A 10 μL aliquot was analyzed for acute cytotoxicity with the trypan blue dye exclusion assay [60] to ensure that cell viability exceeds 70% [61]. The remaining cell suspension was mixed with the same volume of 1% low melting point agarose (LMA), and layered onto clear microscope slides pre-coated with 1% normal melting point agarose. After the first layer of LMA solidified, another layer of 0.5% LMA was applied. Two SCGE microgels were prepared from the suspension harvested from each well. The microgels were immersed in lysing solution (2.5 M NaCl, 1% sodium lauryl sarcosinate, 100 mM Na_2EDTA, 10 mM Tris, pH 10 with 1% Triton X-100 and 10% DMSO) overnight at 4°C.

The DNA of the nuclei in the SCGE microgels was denatured for 20 minutes in electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH 13.5) followed by electrophoresis at 25 V and 300 mA (0.72 V/cm) for 40 minutes at 4°C. After neutralization with 400 mM Tris buffer (pH 7.5) the microgels were dehydrated in cold methanol for 20 minutes, dried for 5 minutes at 50°C, and stored at room temperature. For analysis, the microgels were hydrated in water at 4°C for 20 minutes, and then the nuclei were stained with 65 μL of an ethidium bromide solution (20 μg/mL). Fifty randomly chosen nuclei per microgel were analyzed with a Zeiss fluorescence microscope (excitation filter of BP 546/10 nm, barrier filter of 590 nm) and a computerized image analysis system (Comet Assay IV, Perspective Instruments Ltd., Suffolk, UK). The digitalized data were automatically transferred from the CCD camera to a computer-based spreadsheet for statistical analysis.

3.2.5 Plating efficiency

CHO cells were treated in the identical condition as DNA repair kinetics experiments except that cells were treated in multiple wells concurrently. After 4-hour treatment, the solution was aspirated, and the cells were washed twice with 100 μL HBSS. The cells were harvested with 0.025% trypsin + 0.01% EDTA solution in HBSS. The cell number was counted with Beckman Coulter Z1 Particle Counter (Beckman Coulter, Inc.) and adjusted to 3×10^4 cells/mL. In the new flat-bottom tissue culture 96-well microplate, one column of eight wells was reserved for the blank control consisting of 200 μL of F12+5%

FBS. Titered cells of 100 µL were aliquoted into one column plus 100 µL F12+5% FBS. To prevent volatilization of medium, a sheet of sterile AlumnaSeal™ (RPI Corporation, Mt. Prospect, IL) was pressed over the wells before covering the microplate with a lid. The microplate was placed on a rocking platform for 10 minutes to uniformly distribute the cells, and then was placed in a tissue culture incubator for 72 hours. After incubation, each well was gently aspirated, fixed in 100% methanol for 10 minutes, and stained for 10 minutes with a 1% crystal violet solution in 50% methanol. The plate was gently washed, and 50 µL of a DMSO:methanol (3:1, v/v) solution was added to each well and allowed to contact for 10 minutes. The plate was then analyzed in SpectraMax microplate reader (Molecular Devices, LLC, Sunnyvale, CA) at 595 nm. The blank-corrected absorbency value of the concurrent negative control (cells treated with medium only) was set at 100%. The absorbency for each treatment group well was converted into a percentage of the negative control. For each concentration, eight replicate wells were analyzed per experiment and each experiment was independently repeated at least three times.

3.2.6 Statistical analysis

Each experiment was independently repeated at least three times. The data were transferred to Excel spreadsheets (Microsoft Corp., Redmond, WA) and analyzed using SigmaPlot 11.0 program (Systat Software, Inc. San Jose, CA). The primary measure of DNA damage and repair was the percent of the DNA that had migrated from the nucleus into the microgel (% tail DNA).

The distributions of the % tail DNA values are not normal. In order to conduct parametric statistical analysis the mean % tail DNA value was calculated for each microgel. The mean % tail DNA values for each experimental group were averaged. According to the central limit theorem, these averaged mean values were normally distributed [62]; the data were analyzed using a two-way analysis of variance (ANOVA) test statistic. The mean % tail value from the HAA-treated cells with no recovery time was set at 100% and compared with concurrently treated cells after designated periods of liquid holding. If a significant F value of $P \leq 0.05$ was obtained, a Holm-Sidak multiple-comparison was conducted in order to determine if there was a significant difference in the rate of DNA repair among the monoHANs.

3.3 Results

3.3.1 Genomic DNA damage induction

Dose-response associations have been observed for genomic DNA damage induced by all three monoHANs with the rank order of IAN ≈ BAN >> CAN (Figure 3.1, lower panel) without inducing cytotoxicity (Figure 3.1, upper panel). The data for DNA damage induction were regressed using sigmoidal curve fitting. Based on the concentration-response curves, the HAN concentrations inducing 60 % tail DNA were selected for the experiments on DNA repair kinetics. These concentrations were selected as 60 μM for IAN and BAN, and 1200 μM for CAN (Table 3.1).

3.3.2 DNA repair kinetics

CHO cells were treated in multiple plates concurrently with single concentrations per chemical for 4 hours. After treatment, the cells were washed and the cells from one well were immediately harvested. Fresh serum-free F12 medium was added to the rest of the wells and after different liquid holding times (1, 2, 3, 4, 5, 10 and 24 hours), cells were harvested and SCGE microgels were prepared. The % tail DNA for each HAN at 0 time liquid holding was normalized to a value of 100%. CHO cell viability was determined under liquid holding conditions (serum-free F12 medium) previously and a decline in cell viability was not observed until 40 hours [46]. Thus 24 hours of liquid holding by itself did not cause cytotoxicity. DNA damage induced by HANs was repaired and reduction of genomic DNA damage over time was evaluated with a two-way ANOVA analysis (Figure 3.2, lower panel, Table 3.2). No cytotoxicity was observed throughout the liquid holding DNA repair time (Figure 3.2, upper panel). CAN-induced lesions were repaired most efficiently and the level of unrepaired DNA damage at 3 hour liquid holding was not significantly different from 24-hour liquid holding ($P = 0.066$). The repair process for BAN-induced lesions was less efficient and the unrepaired DNA damage went down to the level at 24-hour liquid holding only after 5 hours; the genomic damage level at 4-hour liquid holding was still statistically different from that for 24-hour liquid holding ($P < 0.001$). IAN-induced lesions were repaired down to the level at 24-hour liquid holding after 4 hours ($P = 0.114$).

The data for each DNA repair curve were regressed using exponential decay fitting. The time required to affect 50% repair of the originally induced DNA damage (t_{50}) was calculated. The t_{50} values for CAN, BAN and IAN were 55, 90 and 81 minutes, respectively.

To determine if there were significant differences in DNA repair at each liquid holding time, a balanced multivariant analysis of variance with a Holm-Sidak pairwise multiple comparison test was conducted (Table 3.3). Significant difference in unrepaired DNA damage level was observed between BAN and IAN ($P = 0.014$) at 1-hour liquid holding, between CAN and BAN, and CAN and IAN at 2-hour liquid holding ($P < 0.001$ for both), between CAN and BAN, and CAN and IAN at 3-hour liquid holding ($P < 0.001$ and $P = 0.011$, respectively), and between CAN and BAN at 4-hour liquid holding ($P = 0.007$).

3.3.3 Frequency distribution of repaired nuclei

The distribution of % tail DNA values of individual nuclei was analyzed as a function of liquid holding time. Figures 3.3 and 3.4 present histograms that illustrate the dynamics of genomic DNA repair with the nucleus as the unit of measure. The 0-hour liquid holding time (Figure 3.3, far left panel of stacked plots) shows that all HANs expressed similar profiles of DNA damage, which confirmed that the selected concentrations of HANs generated approximately similar levels of genomic damage. As the liquid holding time progressed, the levels of DNA damage migrated toward the left in each panel. After 3-hour liquid holding, the level of DNA damage in each treatment group diminished. However, the population of nuclei with higher DNA damage was somewhat more abundant for BAN-treated cells. Few unrepaired nuclei remained after 5 hours (Figure 3.3, two right panels). The DNA damage histogram for the untreated negative controls are presented in Figure 3.4; no difference was observed in DNA damage distribution over 24 hours. With no liquid holding time, the mean of the % tail DNA ± standard error of the mean (SE) was 1.89 ± 0.22, 70.2 ± 3.6, 72.6 ± 3.6 and 61.1 ± 1.6 for negative control, CAN, BAN and IAN, respectively. After 24-hour liquid holding, the mean % tail DNA values were 1.44 ± 0.15, 6.21 ± 0.65, 6.78 ± 0.85 and 7.04 ± 0.53, respectively.

3.3.4 Plating efficiency of HAN-treated cells

The ability of cells to proliferate after exposure to HANs was examined to determine the association of cell growth repressed by cell cycle alteration due to DNA repair. From the CHO cell acute genotoxicity concentration-response curves of HANs (Figure 3.1), HAN concentrations that induced 20%, 40%, and 60% tail DNA values were chosen (IAN at 45 µM, 53 µM, 60 µM, BAN at 43 µM, 52 µM, 60 µM, CAN at 460 µM, 790 µM, 1200 µM) (Table 3.1). To compare these data with previous DNA repair kinetics for HAAs, plating efficiency was also measured for chloroacetic acid (CAA), bromoacetic acid (BAA) and iodoacetic acid (IAA). The HAA concentrations were chosen from those used for the DNA repair kinetic experiments (25 µM for IAA, 60 µM for BAA, and 6 mM for CAA) [46]. Lower and higher concentrations than the concentrations used for DNA repair kinetic study were chosen for comparison as well (15 µM and 35 µM for IAA; 30 µM and 90 µM for BAA; 5 mM and 7 mM for CAA). After the 4-hour cell treatment, the cells were washed and harvested. Equal number of treated cells as well as concurrent negative control cells was plated onto a new 96-well microplate. The resulting cell densities after 72 hours were compared to their negative controls, which was set to 100%. Measurements of the plating efficiency of HAA- and HAN-treated CHO cells are shown in Figures 3.5 and 3.6. Cell growth was suppressed for both groups of DBPs, and revealed a concentration-response relationship. At the chemical concentration used for DNA repair kinetics, the mean ± SE cell density was 56.0 ± 0.9%, 74.4 ± 2.9%, 60.4 ± 4.1%, 64.4 ± 1.5%, 14.6 ± 0.9% and 1.0 ± 0.6% for CAA, BAA, IAA, CAN, BAN and IAN, respectively.

3.4 Discussion

Previously published DNA repair kinetics study with three monoHAAs revealed that different rates of genomic repair was expressed by IAA or CAA versus BAA, which suggested that different DNA lesions and/or different distributions of DNA lesions were induced [46]. The DNA repair kinetics experiments with HANs was conducted to extend the DNA repair kinetic database to a class of N-DBPs. CHO cells were treated with a single concentration per chemical for 4 hours. After the treatment, cells were washed

and the remaining unrepaired DNA was monitored over time. Because the concentration of each HAN was chosen to generate approximately the same level of genotoxic damage, the null hypothesis was that there would be no difference in the DNA repair kinetics if similar DNA lesions were induced by all three HANs.

The association between the rate of DNA repair and the halogen group was not as distinct for the HANs as for the HAAs. A significant difference in unrepaired genomic damage level was observed in BAA-treated cells as compared to IAA and CAA at 1, 2, 3 and 4 hour liquid holding [46]. On the contrary, although some significant differences amongst HANs were observed at specific time periods, the difference was not consistent for an individual HAN (based on the halogen leaving group) as it was observed in HAAs. The combination of BAN and CAN showed the biggest difference in their repair; the unrepaired genomic damage levels were different at 2, 3, and 4 hour liquid holding times between CAN- and BAN-treated cells. The unrepaired genomic damage levels of CAN- and IAN-treated cells were different only at the 3 and 4 hour liquid holding time. The calculated t_{50} vales for CAN, BAN and IAN were 55, 90 and 81 minutes, respectively, whereas those for CAA, BAA, and IAA were 134, 296 and 84 minutes, respectively [46]. The lesions induced by HANs were repaired with greater efficiency as compared to the HAA-induced lesions, which suggested that different types of DNA lesions were induced by the HAAs and the HANs. The HAAs were shown to inhibit glyceraldehyde-3-phosphate dehydrogenase, a key enzyme in glucose metabolism, which may lead to reactive oxygen species formation [63, 64]. BAA-treated cells may have a higher level of double strand DNA breaks [65], which may be associated with the slower rate of repair of BAA-induced DNA lesions.

Another observation that suggests different mode of action by HAAs and HANs is cell morphology after treatment with these chemicals. HAN-treated cells looked round after 4-hour treatment after visual inspection under a microscope but regained normal spindle morphology with time during the liquid holding. HAA-treated cells looked all spindle after the treatment (data now shown). Cell morphology change seemed more severe with the same level of treatment with BAN and IAN than with CAN.

One may question that the washing steps after treatment or liquid holding may remove cells lacking the ability to stick to the surface of the microplate. At each sampling time cells were washed and only adherent cells were collected for DNA repair measurement. Floating cells were observed microscopically at each sampling point for HAN-treated cells. Those cells could be dead or may have been undergoing cell division. Supernatant and cell washing solution were collected into a microcentrifuge tube and cell viability was examined with the trypan blue dye exclusion assay [60]. The number of floating cells was low so that accurate determination of cell viability was difficult. Thus it was concluded that the floating cells were not as abundant as to affect the measured DNA repair rate.

DNA damage induction, DNA repair and cell cycle progression are strongly associated. Cell cycle delay occurs to allow DNA repair to take place [48]. Plating efficiency was measured for HAAs and HANs at the concentration that induces equivalent genomic damage. Cell growth should slow down if cell cycle is severely delayed and faster repair might be associated with cell cycle arrest. At the concentration used for DNA repair kinetics, the suppression of cell growth followed the order of CAA (56.0% of resulting cell density compared to negative control cells) > IAA (60.4%) > BAA (74.4%) for HAAs and IAN (~0%) > BAN (14.6%) > CAN (64.4%) for HANs. The least suppression of cell growth and slow DNA repair rate of BAA-treated cells may indicate that cell cycle was not delayed in BAA-treated cells thus repair for BAA-induced lesions was inefficient. However, a good association of DNA repair kinetics and plating efficiency measurement was not observed for either HAAs or HANs.

Table 3.4 summarizes several relevant physiochemical properties calculated with the Spartan program (www.wavefun.com) for monoHANs and monoHAAs (J. Pals, 2013, unpublished data). Electrophiles (electro deficient) such as HANs form covalent bonds with nucleophilic (electron rich) sites on biological targets. Covalent bond formation between reacting molecules can be described by the properties of their outermost orbitals. The highest energy orbital that contains electrons is called highest occupied molecular orbital (HOMO) and the lowest energy orbital that is vacant is called lowest unoccupied molecular orbital (LUMO) [66]. E_{HOMO} and E_{LUMO} in the entry of Table 3.4 are the frontier molecular orbital energies for those orbitals. Electrophiles and nucleophiles are classified as either soft (relatively polarizable) and hard

(relatively nonpolarizable) and softness (σ) or hardness (η) of certain compound can be calculated using E_{HOMO} and E_{LUMO}. Furthermore, the values for σ and η can be used to calculate the electrophilic index (ω) of the compound, which reflects the tendency of the electrophile to form adducts with a given biological nucleophile [66]. A partition coefficient, log *P*, describes solubility and C-X bond length describes the propensity of halogen leaving group, which are also listed in Table 3.4.

To determine biological mechanisms for monoHAN- and monoHAA-mediated toxicity, multiple correlation analyses were conducted with the physicochemical and toxicological characteristics of these monoHANs using the Pearson's Product Moment test (Table 3.5). The concentrations used for DNA repair kinetic experiments (CAN at 900 µM, BAN at 60 µM, IAN at 60 µM, CAA at 6000 µM, BAA at 60 µM and IAA at 25 µM) were entered for the correlation test (listed as genomic potency in Table 3.5). Mild correlation was observed between t_{50} and log *P* ($r = -0.636$) and between C-X bond length and the genomic potency ($r = -0.650$). Hardness and softness correlated mildly with the genomic potency ($r = 0.678$ and -0.623, respectively) but not with t_{50}. The strongest correlation was observed between t_{50} and the electrophilic index ($r = -0.800$) but the electrophilic index did not correlate with the genomic potency ($r = -0.400$). Electrophiles react with nucleophiles with a significant selectivity, which is determined by their relative softness or hardness. Electrophilic index was successfully associated with electrophile-nucleophile interactions [66]. It is interesting the t_{50} value, not the genomic potency related well with the electrophilic potency. Future research is required to understand this correlation and to extend the DNA repair kinetic database.

In conclusion, HAN-induced DNA lesions were repaired more efficiently than HAA-induced lesions. DNA repair rates were not very different among three HANs regardless of the halogen groups compared to the difference in HAA-treated cells. The lack of an association between DNA repair rate and plating efficiency was confirmed. DNA repair kinetics should be extended to different DBP chemical group, such as haloacetamide and halonitromethanes, and also dihalogenated- and trihalogenated-DBPs. Efforts to elucidate the mechanism of cell cycle alterations by HAAs and HANs are the subject of Chapter 4. These results lead to a detailed analysis of the cell cycle alterations of the HAAs and HANs.

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Tables and Figures

Table 3.1 Concentration of monoHANs that induced equivalent levels of genomic DNA damage in CHO cells.

Compounds	R^2 [a]	SCGE 20% tail DNA (mol/L) [b]	SCGE 40% tail DNA (mol/L) [b]	SCGE 60% tail DNA (mol/L) [b]
Iodoacetonitrile	0.99	4.52×10^{-5}	5.33×10^{-5}	5.94×10^{-5}
Bromoacetonitrile	0.99	4.30×10^{-5}	5.20×10^{-5}	5.94×10^{-5}
Chloroacetonitrile	0.99	4.61×10^{-4}	7.88×10^{-4}	1.19×10^{-3}

[a] R^2 is the coefficient of determination for the regression analysis upon which HAA concentration was calculated for each level of genomic DNA damage. [b]Genotoxicity metric as the percentage of DNA that migrated into the microgel from the nucleus under SCGE conditions. No acute cytotoxicity was observed at any of the monoHAN concentrations used.

Table 3.2 Statistical analysis of the CHO cell DNA repair kinetics data.

Two Way Analysis of Variance Comparison among HAN versus each time point for DNA repair.

General Linear Model

Dependent Variable: SCGE

Source of Variation	DF	SS	MS	F	P
HAN	2	2266.080	1133.040	19.254	<0.001
Time (h)	7	168110.106	24015.729	408.095	<0.001
HAN x Time (h)	14	3076.882	219.777	3.735	<0.001
Residual	184	10828.110	58.848		
Total	207	186721.260	902.035		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of HAN depends on what level of Time (h) is present. There is a statistically significant interaction between HAN and Time (h). (P = <0.001)

Power of performed test with alpha = 0.0500: for HAN : 1.000
Power of performed test with alpha = 0.0500: for Time (h) : 1.000
Power of performed test with alpha = 0.0500: for HAN x Time (h) : 0.992

Least square means for HAN :

Group	Mean	SEM
CAN	28.781	0.858
BAN	35.983	0.959
IAN	35.067	0.959

Least square means for Time (h) :

Group	Mean	SEM
0.000	100.000	1.513
1.000	51.184	1.513
2.000	38.120	1.513
3.000	23.379	1.513
4.000	17.919	1.513
5.000	16.536	1.513
10.000	9.094	1.513
24.000	9.983	1.513

Least square means for HAN x Time (h) :

Group	Mean	SEM
CAN x 0.000	100.000	2.426
CAN x 1.000	48.201	2.426
CAN x 2.000	23.047	2.426
CAN x 3.000	15.639	2.426
CAN x 4.000	13.056	2.426
CAN x 5.000	14.374	2.426
CAN x 10.000	6.640	2.426

Table 3.2 (cont.)

CAN x 24.000	9.289	2.426
BAN x 0.000	100.000	2.712
BAN x 1.000	57.413	2.712
BAN x 2.000	42.651	2.712
BAN x 3.000	29.459	2.712
BAN x 4.000	23.071	2.712
BAN x 5.000	16.084	2.712
BAN x 10.000	10.069	2.712
BAN x 24.000	9.118	2.712
IAN x 0.000	100.000	2.712
IAN x 1.000	47.940	2.712
IAN x 2.000	48.663	2.712
IAN x 3.000	25.039	2.712
IAN x 4.000	17.631	2.712
IAN x 5.000	19.150	2.712
IAN x 10.000	10.573	2.712
IAN x 24.000	11.540	2.712

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: **Time (h) within CAN**

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
0.000 vs. 10.000	93.360	27.213	<0.001	0.002	Yes
0.000 vs. 24.000	90.711	26.441	<0.001	0.002	Yes
0.000 vs. 4.000	86.944	25.343	<0.001	0.002	Yes
0.000 vs. 5.000	85.626	24.959	<0.001	0.002	Yes
0.000 vs. 3.000	84.361	24.590	<0.001	0.002	Yes
0.000 vs. 2.000	76.953	22.431	<0.001	0.002	Yes
0.000 vs. 1.000	51.799	15.099	<0.001	0.002	Yes
1.000 vs. 10.000	41.561	12.114	<0.001	0.002	Yes
1.000 vs. 24.000	38.911	11.342	<0.001	0.003	Yes
1.000 vs. 4.000	35.144	10.244	<0.001	0.003	Yes
1.000 vs. 5.000	33.827	9.860	<0.001	0.003	Yes
1.000 vs. 3.000	32.561	9.491	<0.001	0.003	Yes
1.000 vs. 2.000	25.154	7.332	<0.001	0.003	Yes
2.000 vs. 10.000	16.407	4.782	<0.001	0.003	Yes
2.000 vs. 24.000	13.758	4.010	<0.001	0.004	Yes
2.000 vs. 4.000	9.991	2.912	0.004	0.004	No
3.000 vs. 10.000	8.999	2.623	0.009	0.004	No
2.000 vs. 5.000	8.673	2.528	0.012	0.005	No
5.000 vs. 10.000	7.734	2.254	0.025	0.005	No
2.000 vs. 3.000	7.408	2.159	0.032	0.006	No
4.000 vs. 10.000	6.416	1.870	0.063	0.006	No
3.000 vs. 24.000	6.350	1.851	0.066	0.007	No
5.000 vs. 24.000	5.085	1.482	0.140	0.009	No
4.000 vs. 24.000	3.767	1.098	0.274	0.010	No
24.000 vs. 10.000	2.649	0.772	0.441	0.013	No
3.000 vs. 4.000	2.583	0.753	0.452	0.017	No
5.000 vs. 4.000	1.318	0.384	0.701	0.025	No
3.000 vs. 5.000	1.265	0.369	0.713	0.050	No

Table 3.2 (cont.)

Comparisons for factor: **Time (h) within BAN**

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
0.000 vs. 24.000	90.882	23.694	<0.001	0.002	Yes
0.000 vs. 10.000	89.931	23.446	<0.001	0.002	Yes
0.000 vs. 5.000	83.916	21.878	<0.001	0.002	Yes
0.000 vs. 4.000	76.929	20.056	<0.001	0.002	Yes
0.000 vs. 3.000	70.541	18.391	<0.001	0.002	Yes
0.000 vs. 2.000	57.349	14.952	<0.001	0.002	Yes
1.000 vs. 24.000	48.294	12.591	<0.001	0.002	Yes
1.000 vs. 10.000	47.344	12.343	<0.001	0.002	Yes
0.000 vs. 1.000	42.587	11.103	<0.001	0.003	Yes
1.000 vs. 5.000	41.329	10.775	<0.001	0.003	Yes
1.000 vs. 4.000	34.342	8.953	<0.001	0.003	Yes
2.000 vs. 24.000	33.532	8.742	<0.001	0.003	Yes
2.000 vs. 10.000	32.582	8.494	<0.001	0.003	Yes
1.000 vs. 3.000	27.953	7.288	<0.001	0.003	Yes
2.000 vs. 5.000	26.567	6.926	<0.001	0.004	Yes
3.000 vs. 24.000	20.341	5.303	<0.001	0.004	Yes
2.000 vs. 4.000	19.580	5.105	<0.001	0.004	Yes
3.000 vs. 10.000	19.390	5.055	<0.001	0.005	Yes
1.000 vs. 2.000	14.762	3.849	<0.001	0.005	Yes
4.000 vs. 24.000	13.952	3.638	<0.001	0.006	Yes
3.000 vs. 5.000	13.375	3.487	<0.001	0.006	Yes
2.000 vs. 3.000	13.191	3.439	<0.001	0.007	Yes
4.000 vs. 10.000	13.002	3.390	<0.001	0.009	Yes
4.000 vs. 5.000	6.987	1.822	0.070	0.010	No
5.000 vs. 24.000	6.966	1.816	0.071	0.013	No
3.000 vs. 4.000	6.389	1.666	0.097	0.017	No
5.000 vs. 10.000	6.015	1.568	0.119	0.025	No
10.000 vs. 24.000	0.951	0.248	0.805	0.050	No

Comparisons for factor: **Time (h) within IAN**

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
0.000 vs. 10.000	89.427	23.315	<0.001	0.002	Yes
0.000 vs. 24.000	88.460	23.063	<0.001	0.002	Yes
0.000 vs. 4.000	82.369	21.475	<0.001	0.002	Yes
0.000 vs. 5.000	80.850	21.079	<0.001	0.002	Yes
0.000 vs. 3.000	74.961	19.543	<0.001	0.002	Yes
0.000 vs. 1.000	52.060	13.573	<0.001	0.002	Yes
0.000 vs. 2.000	51.337	13.384	<0.001	0.002	Yes
2.000 vs. 10.000	38.090	9.931	<0.001	0.002	Yes
1.000 vs. 10.000	37.367	9.742	<0.001	0.003	Yes
2.000 vs. 24.000	37.123	9.679	<0.001	0.003	Yes
1.000 vs. 24.000	36.400	9.490	<0.001	0.003	Yes
2.000 vs. 4.000	31.032	8.090	<0.001	0.003	Yes
1.000 vs. 4.000	30.309	7.902	<0.001	0.003	Yes
2.000 vs. 5.000	29.513	7.694	<0.001	0.003	Yes
1.000 vs. 5.000	28.790	7.506	<0.001	0.004	Yes
2.000 vs. 3.000	23.624	6.159	<0.001	0.004	Yes
1.000 vs. 3.000	22.901	5.971	<0.001	0.004	Yes
3.000 vs. 10.000	14.466	3.771	<0.001	0.005	Yes
3.000 vs. 24.000	13.499	3.519	<0.001	0.005	Yes

Table 3.2 (cont.)

5.000 vs. 10.000	8.577	2.236	0.027	0.006	No
5.000 vs. 24.000	7.610	1.984	0.049	0.006	No
3.000 vs. 4.000	7.408	1.931	0.055	0.007	No
4.000 vs. 10.000	7.058	1.840	0.067	0.009	No
4.000 vs. 24.000	6.091	1.588	0.114	0.010	No
3.000 vs. 5.000	5.889	1.535	0.126	0.013	No
5.000 vs. 4.000	1.519	0.396	0.693	0.017	No
24.000 vs. 10.000	0.967	0.252	0.801	0.025	No
2.000 vs. 1.000	0.724	0.189	0.851	0.050	No

Table 3.3 Two-way Analysis of Variance for DNA repair of CHO cells treated with IAN, BAN or CAN (Holm-Sidak pairwise multiple comparison).

Comparison	Liquid Holding Time (h)	Difference of means	*P*
CAN vs BAN	0	0	1
CAN vs IAN	0	1.28×10^{-13}	1
BAN vs IAN	0	1.28×10^{-13}	1
CAN vs BAN	1	9.21	0.012
CAN vs IAN	1	0.26	0.943
BAN vs IAN *	1	9.47	0.014
CAN vs BAN *	2	19.60	<0.001
CAN vs IAN *	2	25.62	<0.001
BAN vs IAN	2	6.01	0.119
CAN vs BAN *	3	13.82	<0.001
CAN vs IAN *	3	9.40	0.011
BAN vs IAN	3	4.42	0.251
CAN vs BAN *	4	10.01	0.007
CAN vs IAN	4	4.58	0.21
BAN vs IAN	4	5.44	0.158
CAN vs BAN	5	1.71	0.639
CAN vs IAN	5	4.78	0.191
BAN vs IAN	5	3.07	0.425
CAN vs BAN	10	3.43	0.347
CAN vs IAN	10	3.93	0.281
BAN vs IAN	10	0.50	0.896
CAN vs BAN	24	0.17	0.963
CAN vs IAN	24	2.25	0.537
BAN vs IAN	24	2.42	0.529

* DNA repair levels were statistically different between the treatment groups at $P < 0.05$.

Table 3.4 Summary of physicochemical properties of monohaloacetonitriles and monohaloacetic acids *.

Compound	Chemical Formula	C-X Length (Å)	log *P*	E_{HOMO}	E_{LUMO}	Hardness η (ev)	Softness σ (ev^{-1})	Electrophilic index ω (ev)
Chloroacetonitrile	$ClCH_2CN$	1.81	1.04	-12.4	3.64	8.02	0.125	1.196
Bromoacetonitrile	$BrCH_2CN$	1.96	1.16	-11.23	3.23	7.23	0.138	1.107
Iodoacetonitrile	ICH_2CN	2.18	1.55	-10.03	2.03	6.03	0.166	1.327
Chloroacetic acid	$ClCH_2COOH$	1.78	0.22	-12.17	4.67	8.42	0.119	0.835
Bromoacetic acid	$BrCH_2COOH$	1.93	0.34	-11.05	4.28	7.67	0.130	0.747
Iodoacetic acid	ICH_2COOH	2.16	0.73	-9.9	2.7	6.30	0.159	1.029

* Unpublished data from J. Pals, 2013.

Table 3.5 Pearson correlation analysis of physicochemical and toxicological measurements of monohaloacetonitriles.

	t_{50} (min)	Genomic potency (μM)
C-X bond length	-0.191	-0.650
log *P*	-0.636	-0.570
E_{HOMO}	-0.00458	-0.618
E_{LUMO}	0.553	0.655
Hardness	0.288	0.678
Softness	-0.311	-0.623
Electrophilic index	-0.800	-0.400
t_{50}	-	-0.0125

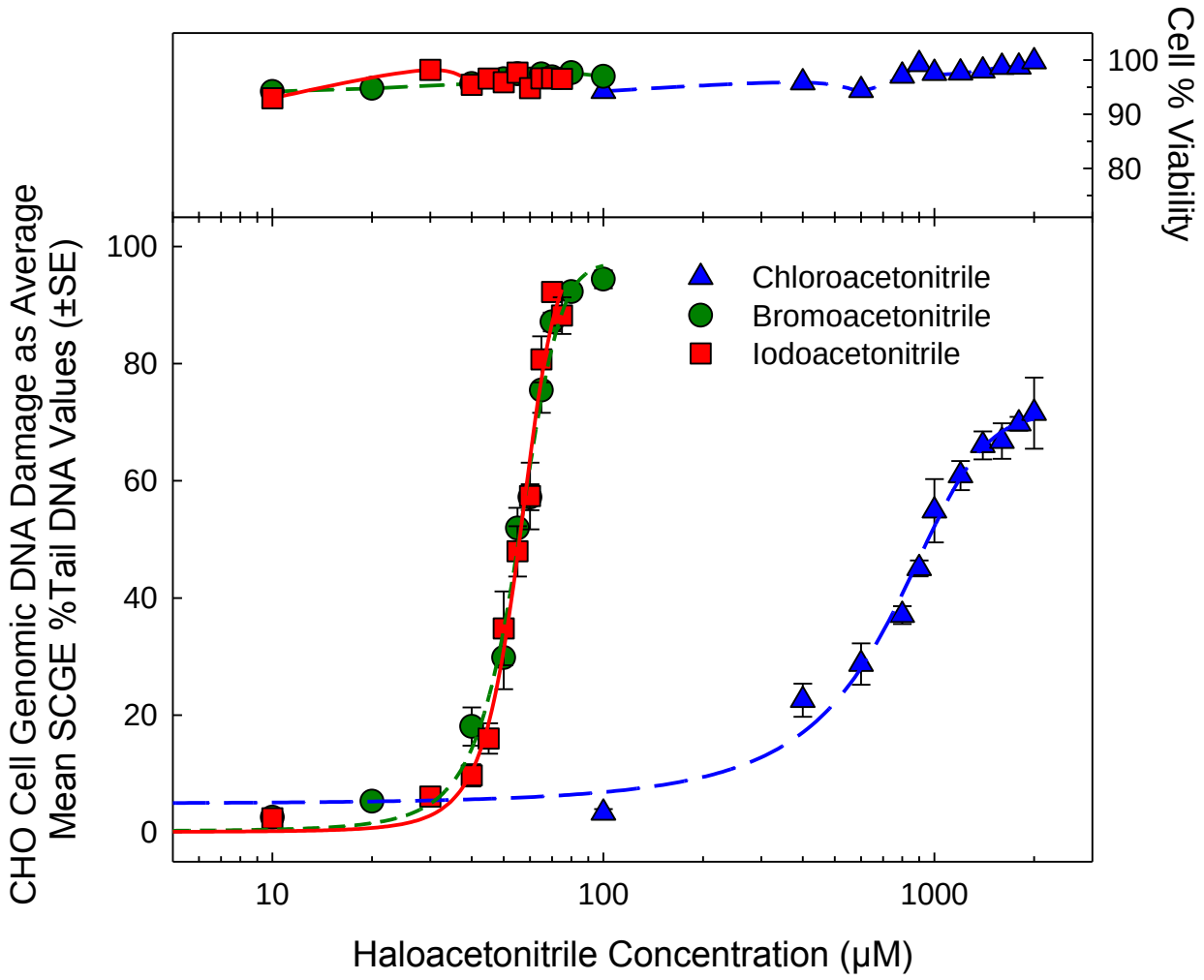

Figure 3.1 Genotoxicity of CAN, BAN, and IAN in the SCGE assay in CHO cells after 4 hours of treatment (lower panel) and the cell viability (top panel).

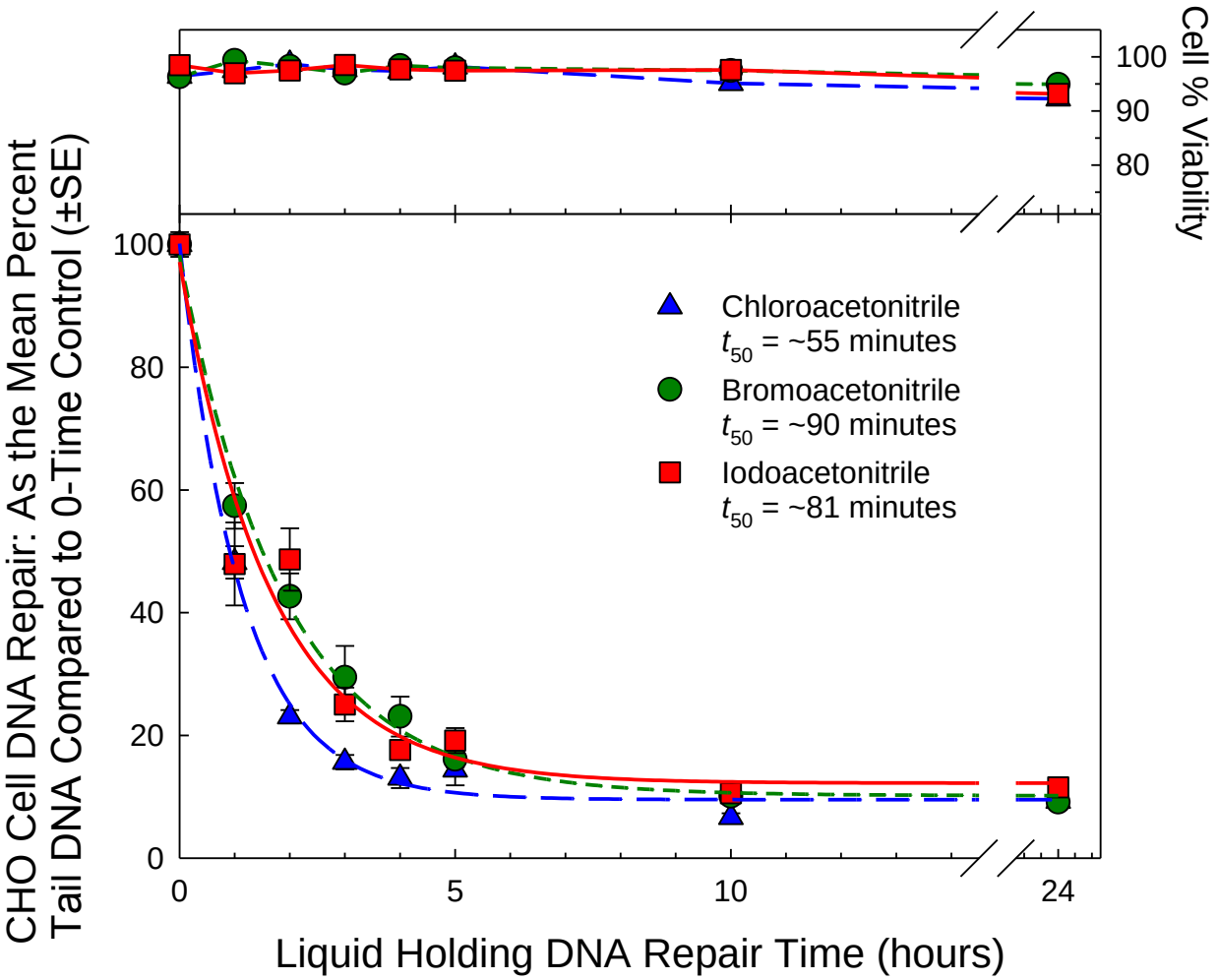

Figure 3.2 DNA repair kinetics in CHO cells treated with CAN (1200 µM), BAN (60 µM) and IAN (60 µM) for 4 hours and then maintained in fresh serum-free F12 medium for 0, 1, 2, 3, 4, 5, 10 and 24 hours (lower panel). Cell viability (top panel).

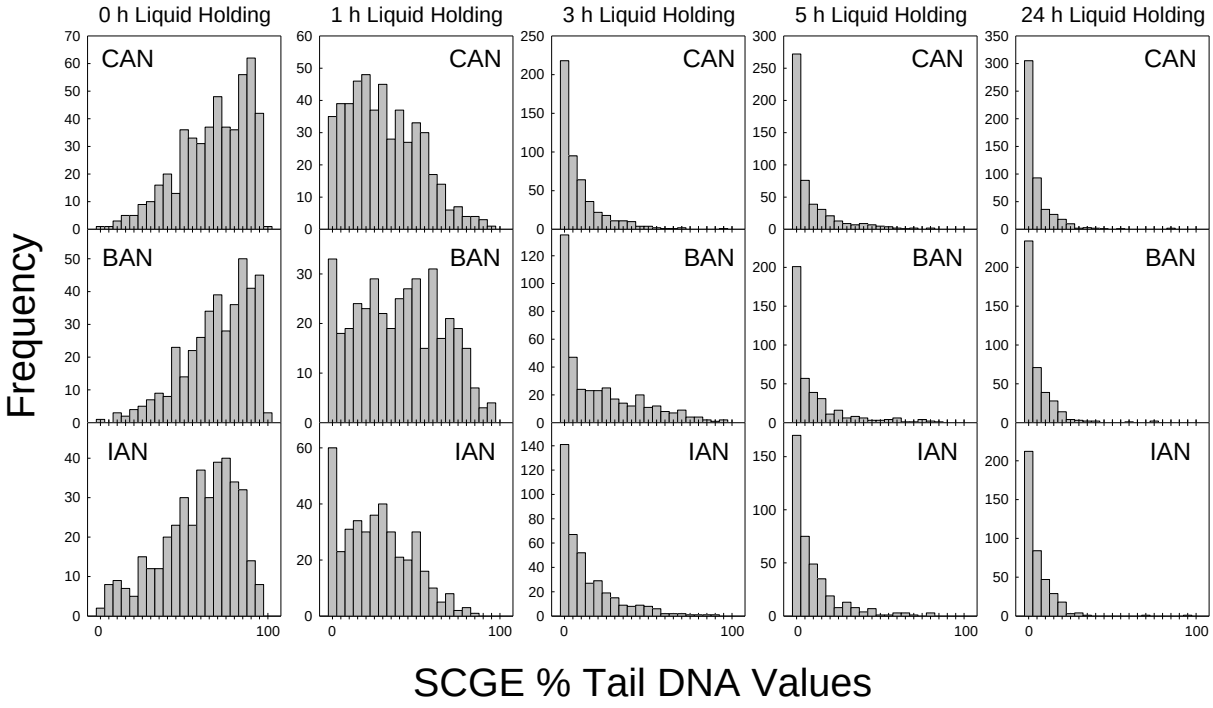

Figure 3.3 Histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with chloroacetonitrile (CAN), bromoacetonitrile (BAN), and iodoacetonitrile (IAN) without and with 1, 3, 5 and 24 hour liquid holding recovery time.

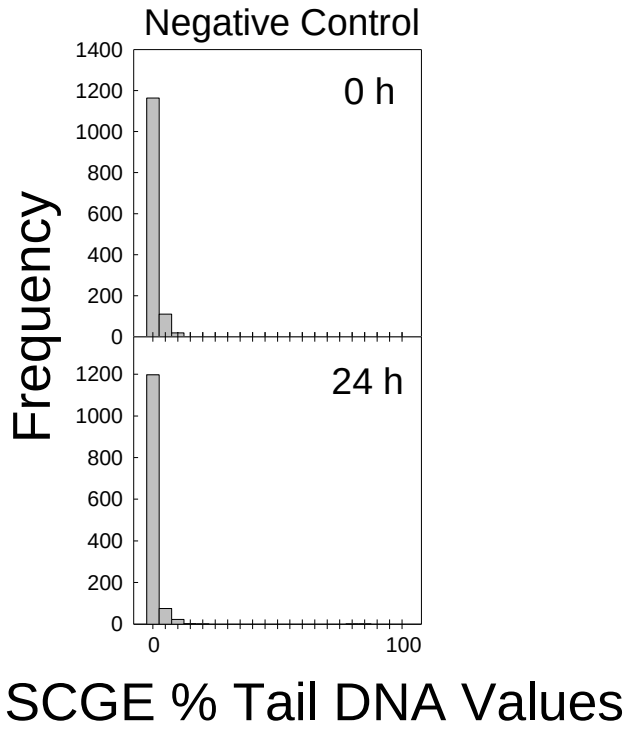

Figure 3.4 Histograms illustrating the distributions of SCGE % tail DNA values from the CHO cell negative controls at 0 and 24 hour liquid holding recovery time.

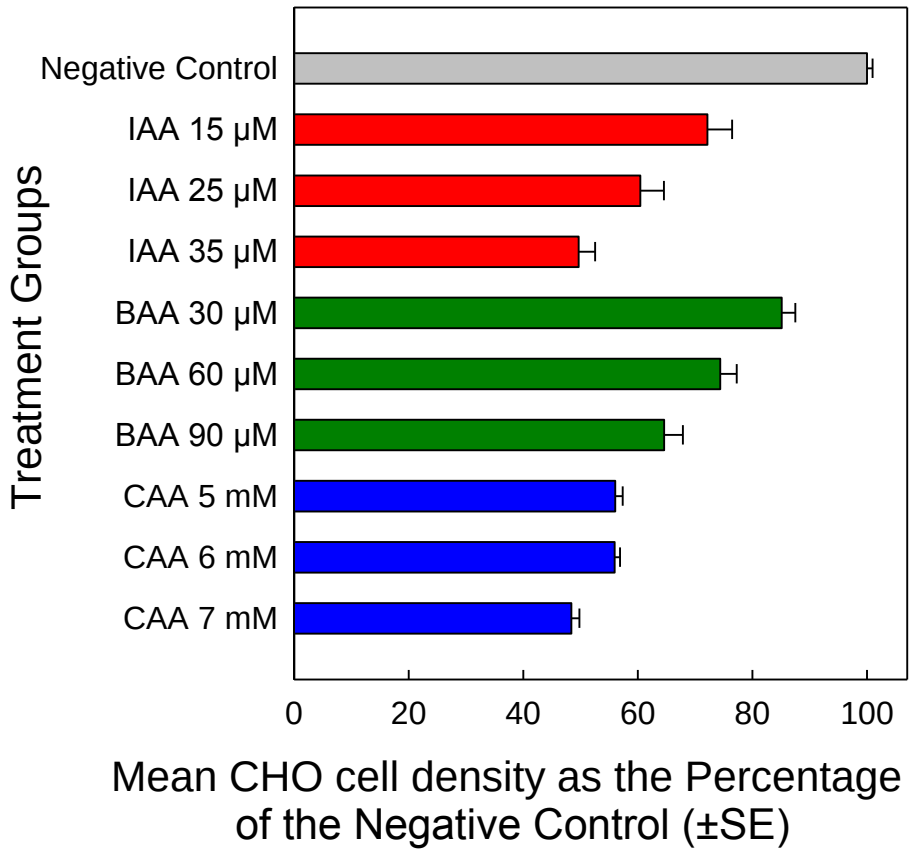

Figure 3.5 Plating efficiency of HAA-treated CHO cells.

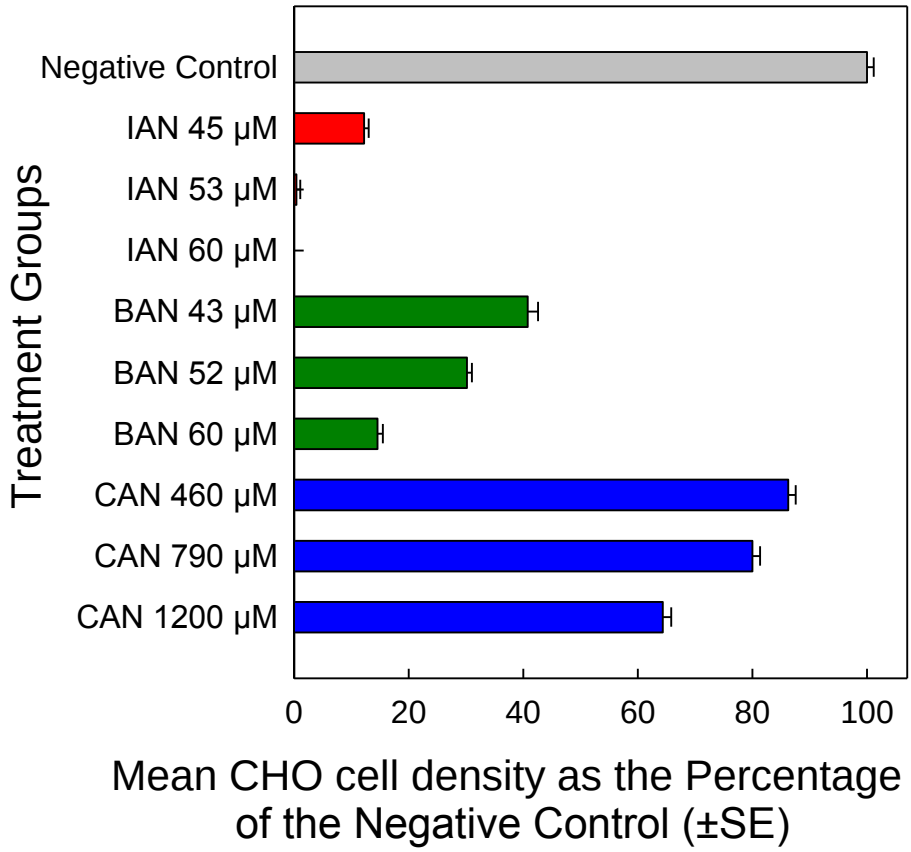

Figure 3.6 Plating efficiency of HAN-treated CHO cells.

CHAPTER 4

ANALYSES OF CELL CYCLE ALTERATION INDUCED BY HALOACETONITRILES

ABSTRACT

Haloacetonitriles (HANs) are a chemical class of disinfection byproducts (DBPs) that form from reactions between disinfectants and dissolved nitrogen precursors, the latter more prevalent in water sources impacted by algae bloom and municipal wastewater effluent discharge. HANs were demonstrated to be genotoxic, thus subsequent effects in mammalian cell cycle was investigated. Treating Chinese hamster ovary (CHO) cells with monoHANs followed by the release from the treatment resulted in abnormally high DNA content over time. It appeared that HANs adversely affected the cell cycle progression by interfering with cell division. Presumably HAN-treated cells underwent another cell cycle and became tetraploid, and further progression of cell cycle resulted in induction of high ploidy cells. There are accumulating evidences of transient tetraploid state proceeding aneuploidy in cancer progression. It is possible that HAN disruption of the normal cell cycle and the generation of aberrant cells with abnormal number of chromosomes may contribute to cancer induction and adverse health outcomes associated with consumption of disinfected water. This is the first demonstration of the induction of high ploidy by a class of DBPs.

4.1 Introduction

Water disinfection results in the formation of environmentally hazardous chemicals called disinfection by-products (DBPs). Exposure to DBPs is associated with urinary bladder cancer and adverse pregnancy outcomes [1]. Haloacetonitriles (HANs) are a chemical class of DBPs that form from reactions between disinfectants (e.g., chloramines) and dissolved nitrogen precursors (e.g., amino acids) [2-6]. The research activity into nitrogenous DBPs (N-DBPs), in which HANs are categorized, has increased partly because recent *in vitro* mammalian cell tests have shown that the HANs, haloacetamide and

halonitromethanes are all far more cyto- and genotoxic than the regulated trihalomethanes (THMs) and haloacetic acids (HAAs) [7-10].

Population growth and rising water supply demand is putting stress on water sources availability; water providers are increasingly using water sources which are impacted by algae bloom and municipal wastewater effluent discharge, which may promote N-DBP formation due to elevated levels of organic nitrogen content [5, 6, 11-13]. Utilities are exploring new disinfectant combinations to reduce the formation of regulated DBPs but some alternative disinfection schemes may promote N-DBP formation [5]. HANs are not currently regulated by U.S. Environmental Protection Agency (EPA), but the World Health Organization set the guideline value for dichloroacetonitrile (DCAN) and dibromoacetonitrile (DBAN) at 20 µg/L and 70 µg/L, respectively [14]. The major species of HANs are DCAN, trichloroacetonitrile (TCAN), DBAN and bromochloroacetonitrile (BCAN) and these species are sometimes referred as HAN4. HANs measured in the nationwide occurrence studies of 12 U.S. water treatment plants ranged up to 14 µg/L (median: 3 µg/L) [15]. HANs were also measured in chlorinated municipal wastewater treatment plant effluents (median: 16 µg/L) as well as in chloraminated wastewater effluents (median: 0.3 µg/L). Recently HANs were shown to form as an electrochemical oxidation by-product of landfill leachate [16]. HANs can also form in biological systems *in vivo* from residual hypochlorite in drinking water [17]. HAN formation in swimming pool is reported, where HANs can be absorbed through dermal exposure [18, 19].

Several HANs are known to interact with DNA molecules [20] and also are mutagenic in *Salmonella typhimurium* [21-23], genotoxic in mammalian cells [8, 22, 23], clastogenic [24] and developmentally toxic [25, 26]. DCAN, but not DBAN, was shown to be an effective inducer of aneuploidy in oocysts of *Drosophila melanogaster* [27]. The metabolism and toxicity of HANs have been reviewed extensively [28]. DBAN was recently shown to be carcinogenic [29] and its contribution to lifetime risk of cancer by HANs was calculated to be approximately 3 – 10× as potent as trihalomethanes (THMs), a regulated class of DBPs, using quantitative structural toxicity relationship program [30]. Chloroacetonitrile (CAN) and/or

its metabolites were shown to cross the placenta and accumulate in fetal brain tissues in mice where it caused oxidative stress and neural apoptosis [31, 32].

While broad range of toxicological data is available in the literature, they often times center around chlorinated and brominated HANs. Systematic genotoxicity study including iodinated HANs has not been published until 2007; *in vitro* mammalian cell toxicity demonstrated a descending rank order of dibromoacetonitrile (DBAN) > bromoacetonitrile (BAN) ≈ iodoacetonitrile (IAN) > BCAN > DCAN > CAN > TCAN for chronic cytotoxicity, and IAN > BAN ≈ DBAN > BCAN > CAN > TCAN > DCAN for acute genotoxicity [8]. The comparative, quantitative systematic cyto- and genotoxicity study on halogenated DBPs using a non-neoplastic Chinese hamster ovary (CHO) cells revealed a rank order of cyto- and genotoxicity following the rank order of iodinated DBPs (I-DBPs) > brominated DBPs (Br-DBPs) > chlorinated DBPs (Cl-DBPs). HAN cyto- and genotoxicity in CHO cells follow the same halogen order [8] as well as HAAs [33], for which molecular mechanism of toxic action has associated the genotoxicity induction with propensity of the halogen to leave the group [34] (Figure 4.1).

DNA damage in eukaryotic cells is strongly associated with cell cycle modulation. A damage response pathway triggered by genetic insults regulates cell cycle arrest and apoptosis (programmed cell death) as well as controls the activation of DNA repair pathways [35]. Eukaryotic cell cycle consists of interphase (G_0/G_1, S, and G_2) and mitotic (M) phase (Figure 4.2 (A)). Each chromosome is duplicated during S phase (2N→4N; N values denote DNA content as a multiple of the normal haploid genome); cells in G_2 phase have four copies of chromosomes (4N) whereas cells in G_1 phase have two copies (2N). Cells divide in M phase, where chromosome condensation, chromosome alignment and separation, daughter cell separation (cytokinesis) occur (Figure 4.2 (B)). Progress of cell cycle is strictly monitored to ensure the genome integrity and correct separation of newly replicated sister chromatids. Nuclear DNA must be duplicated once and only once at each cell cycle [36]. The cell cycle is controlled in a directional and sequential pattern by cell cycle regulators, complexes of proteins called cyclins and cyclin-dependent kinases (Cdks). These cyclin:Cdk complexes are responsible for promoting cell survival by protecting the cell from replicating damaged DNA or from mutations that may lead to aberrant and uncontrolled cell

growth (neoplasia). Each cyclin:Cdk complex in the cell cycle acts as a checkpoint and can halt progression through the cell cycle and either arrest progression or induce apoptosis [37-40]. Since HANs are mutagenic, genotoxic and clastogenic, they are likely to affect mammalian cell cycle. There is no systematic analysis of the cell cycle alteration by a class of DBPs in the literature, with all three halogens included (I, Br and Cl). In this study, CHO cells are treated with CAN, BAN and IAN and subsequent cell cycle profile was monitored. Flow cytometry was used to measure the cell cycle distribution, combined with plating efficiency measurement and DNA strand break analysis. Flow cytometric analysis is a widely used technique to monitor the progression of cell cycle by quantifying fluorescent dye incorporation to DNA [41, 42]. The intensity of the fluorescence is recorded at a single cell level and the obtained frequency distribution represents cells with different DNA content based on the cell cycle progression. The objective of this research was to determine if HANs could alter the cell cycle of treated mammalian cells.

4.2 Materials and Methods

4.2.1 Reagents

General reagents were purchased from Fisher Scientific Co. (Itasca, IL) and Sigma-Aldrich Co. (St. Louis, MO). Iodoacetonitrile (98%, CAS# 624-75-9), bromoacetonitrile (97%, CAS# 590-17-0) and chloroacetonitrile (99%, CAS# 107-14-2) were purchased from Sigma-Aldrich. HANs were diluted in dimethyl sulfoxide (DMSO) at 1 mol/L and stored at −22°C. Ethylmethane sulfonate (EMS, CAS# 62-50-0) was diluted in DMSO at 2.5 mol/L and stored at −22°C. Cytosine-β-D-arabino-furanoside hydrochloride (AraC, CAS# 64-74-9) was dissolved in Ham's F12 medium at 100 mM and stored at −22°C. Propidium iodide (95%, CAS# 25535-16-4) was purchased from Acros Organics (Morris Plains, NJ) and dissolved in sterile distilled-deionized water at 1.0 mg/mL. RNase A (20 mg RNase A/mL in 50 mM Tris-HCl (pH 8.0), 10 mM EDTA) was purchased from Life Technologies (Grand Island, NY).

4.2.2 Cells and culture media

Transgenic Chinese hamster ovary (CHO) cells line AS52 clone 11-4-8 [43, 44] were maintained in modified Ham's F12 medium (Mediatech, Inc., Manassas, VA) supplemented with 5% fetal bovine serum (FBS), 1% L-glutamine and 1% antibiotic-antimycotic solution (10 units/mL penicillin G sodium, 10 µg/mL streptomycin sulfate, 25 µg/mL amphotericin B, 0.85% saline; Invitrogen, Carlsbad, CA). The cells exhibit normal morphology, express cell contact inhibition and grow as a monolayer without expressing neoplastic foci. The cells were grown in 100-mm glass culture plate or in 75-cm^2 tissue culture flask at 37°C in a humidified atmosphere of 5% CO_2. Cell doubling time was 14 hours.

4.2.3 Cell treatment

Unsynchronized CHO cells were plated in sterile flat-bottom 6-well tissue culture plates at 2×10^5 cells/well/(2 mL F12+5% FBS). When the post-incubation was longer than 26 hours, initial cell loading was reduced to 5×10^4 cells/well/(2 mL of F12+5% FBS) for the negative control. After 16 – 20 hours of incubation, cells were rinsed twice with 1 mL of Hank's balanced salt solution (HBSS) without divalent cations, and treated with each chemical in 1 mL of serum-free F12 for 4 hours at 37°C, 5% CO_2. Multiple wells were used per treatment group as needed. A sheet of sterile AlumnaSeal™ (RPI Corporation, Mt. Prospect, IL) was pressed over the wells before covering the plate with a lid to prevent crossover contamination between wells due to volatilization.

4.2.4 Flow cytometric analysis of cell cycle

After the 4-hour treatment, the solution was aspirated, and the cells were washed twice with 1 mL HBSS. The cells were harvested immediately (0 hour sample) with 0.5× trypsin (0.05% trypsin, in HBSS with 0.2 g/L EDTA, without calcium, magnesium, Hyclone Laboratories, South Logan, UT), or 2 mL of drug-free F12+5% FBS were added and incubated for designated times. The post-treatment incubation times allowed cells to accumulate at intervals where there was cell cycle blockage. Harvested cells were counted with Beckman Coulter Z1 Particle Counter (Beckman Coulter, Inc., Brea CA), and acute

cytotoxicity was determined using the trypan blue dye exclusion assay [45]. In order to determine their DNA content, harvested cells were fixed in 70% ice cold ethanol. Samples were stored for up to one week at −22°C. The fixed cells were rinsed with cold phosphate buffered saline (PBS) twice and then incubated for 30 minutes at 37°C with 50 µg/mL of the DNA fluorochrome propidium iodide, a marker of DNA content, in a solution containing 200 µg/mL of RNase A. Cell fluorescence was measured with a Biosciences LSR II flow cytometry analyzer (San Jose, CA). Propidium iodide was excited by a 488 nm laser line (laser diode) and fluorescence emission was collected through 695/40 nm band-pass filter. The data were analyzed using multicycle DNA analysis using FCS Express version 4 (De Novo Software, Los Angeles, CA) and aggregated cells were gated out.

4.2.5 Cell visualization

For microscopic investigation of cell morphology, cells were treated as described above and adherent cells at 0, 26, and 52 hours of post-incubation were imaged using a 10× objective with phase contrast in an inverted microscope (Olympus, Center Valley, PA). Alternatively, cells at 0, 26, and 52 hours of post-incubation were harvested and closer inspection images of the suspended cells were taken using a 40× bright field illumination objective. The resulting images were saved as 8-bit grayscale files in TIFF format.

4.2.6 Plating efficiency

After a 4-hour treatment period, the solution was aspirated, and cells were washed twice with 1 mL HBSS. The cells were then harvested with 0.5× trypsin (0.025% trypsin+0.1 g/L EDTA). The cell number was counted with Beckman Coulter Z1 Particle Counter and adjusted to 3×10^4 cells/mL. In a flat-bottom, tissue culture 96-well microplate, one column of eight wells was reserved for the blank control consisting of 200 µL of F12+5% FBS. Titered cells of 100 µL were aliquoted into one column plus 100 µL F12+5% FBS. To prevent volatilization of medium, a sheet of sterile AlumnaSeal™ (RPI Corporation, Mt. Prospect, IL) was pressed over the wells before covering the microplate with a lid. The microplate was

placed on a rocking platform for 10 minutes to uniformly distribute the cells, and then was placed in a tissue culture incubator for 72 hours. After incubation, each well was gently aspirated, fixed in 100% methanol for 10 minutes, and stained for 10 minutes with a 1% crystal violet solution in 50% methanol. The plate was gently washed, and 50 µL of a DMSO:methanol (3:1, v/v) solution was added to each well and allowed to contact for 10 minutes. The plate was then analyzed in SpectraMax microplate reader (Molecular Devices, LLC, Sunnyvale, CA) at 595 nm. The blank-corrected absorbency value of the negative control was set at 100%. The absorbency for each treatment group well was converted into a percentage of the negative control. For each concentration, eight replicate wells were analyzed per experiment and each experiment was independently repeated at least three times.

4.2.7 DNA strand break analysis using single cell gel electrophoresis

Cells at 0 and 26 hour post-incubation were harvested and analyzed for DNA strand breaks. Briefly, after HBSS rinse, cells were detached with 400 µL of 0.5× trypsin (0.025% trypsin+0.1 g/L EDTA) followed by the addition of 300 µL F12+5% FBS. A 120 µL of cell suspension was mixed with 120 µL of 1% low melting point agarose (LMA) in PBS, and 90 µL of the mixture was applied onto clear microscope slides that were pre-coated with a layer of 1% normal melting point agarose prepared in deionized water. After the first LMA layer was solidified, another layer of 0.5% LMA was applied. Two microgels were prepared from each treatment group. Acute cytotoxicity was also determined to ensure that cell viability was over 70% [46]. The cellular membrane and cytoplasm as well as almost all histones were removed by an overnight immersion in lysing solution (2.5 M NaCl, 1% sodium lauryl sarcosinate, 100 mM Na_2EDTA, 10 mM Tris, pH 10 with 1% Triton X-100, and 10% DMSO) at 4°C. The microgels were placed in alkaline buffer (1 mM EDTA and 300 mM NaOH, pH 13.5), where DNA were denatured for 20 minutes followed by electrophoresis at 25 V and 300 mA (0.72 V/cm) for 40 minutes at 4°C. The microgels were neutralized in 400 mM Tris buffer (pH 7.5), dehydrated in cold methanol, dried at 50°C and stored in a covered slide box at room temperature. For analysis, the microgels were rehydrated in cold water and stained with 65 µL of 20 µg/mL ethidium bromide solution. Individual nuclei were analyzed

with a Zeiss fluorescence microscope (excitation filter of BP 546/10 nm, barrier filter of 590 nm) integrated with a CCD camera. Digitized image was analyzed with Comet Assay IV (Perceptive Instruments Ltd., Suffolk, UK). The percentage of DNA in the tail (% tail DNA) is the primary measurement as the DNA genomic damage since it bears a linear relationship to break frequency, and is relatively unaffected by threshold setting [47, 48].

4.3 Results

The initial loading of cells was determined based on the cell doubling time of 14 hours (data not shown). Since CHO cells grow as a monolayer, they exert cell contact inhibition when they become confluent. Xenobiotics can exhibit different cell-cycle specificities in different cell growing cell status [49] thus care was taken when optimizing the initial cell loading so that cell cycle arrest was not induced by cell contact inhibition. With the initial cell density of 2×10^5 cells/well, negative control cells could grow exponentially up to 26 hours of post-incubation. When the post-incubation exceeded 26 hours, initial cell loading for negative control was reduced to 5×10^4 cells/well. The cell loading for HAN treatment group was kept at 2×10^5 cells/well since the cell number did not increase for the first 26 hours of post-incubation (discussed below).

4.3.1 Optimization of duration of post-incubation

The duration of post-incubation after 4 hour treatment was first optimized using AraC as a positive control. AraC is a dideoxy-analogue of nucleoside, which has been used clinically as an antiviral and antitumor agent. The major intracellular form of AraC is the triphosphate derivative (AraCTP) and it inhibits DNA synthesis by being incorporated into nucleic acids, particularly DNA. The replacement of dCTP by AraCTP leads to termination of almost all polynucleotide chain after the incorporation of the analogue [50]. Since cells cannot divide upon termination of DNA strand elongation, AraC is a good cell cycle arrest inducer [49, 51]. In order to observe the cell cycle arrest, cells that have halted at certain cell cycle checkpoint needs to accumulate. Cell cycle profile of AraC-treated CHO cells was remarkably

different compared to that of negative control CHO cells (Figure 4.3). Cell cycle distribution is displayed as 2N, 2N-4N, and 4N instead of G_1, S, G_2/M since flow cytometric analysis using propidium iodide cannot distinguish if 4N cells are diploid (baseline is two pairs of chromosomes; this is the normal state) at G_2/M or tetraploid cells (baseline is four pairs of chromosome; usually the consequence of failure in cell division) at G_1 [41]. AraC 100 µM effectively induced cell cycle blockages, and 4N at 14, 20, 26 hour of post-incubation for AraC-treated cells was 90.1%, 59.7%, and 78.4%, respectively, whereas 4N at 14, 20, 26 hour of post-incubation for negative control cells was 30.7%, 13.3%, and 14.7%, respectively. AraC 50 µM also resulted in comparable cell cycle modulation and shifted the cell cycle toward 4N. Based on the comparison of cell cycle distribution between negative control cells and AraC-treated cells, 26 hour was determined to be the observation window.

4.3.2 Optimization of HAN concentration

Preliminary experiments demonstrated that CHO cells treated with HAN concentrations of 1200 µM CAN, 60 µM BAN, and 60 µM IAN, as was used for DNA repair kinetics study (Chapter 3), caused excessive cytotoxicity after 26 hours of post-incubation. Treatment concentration was optimized empirically so that cell cycle blockage would be induced without causing excessive cytotoxicity. CHO cells were treated with incremental HAN concentrations for 4 hours and left in fresh F12+5% FBS for 26 hours. Cell cycle alternation became prominent with increasing concentrations (Figure 4.4) without excessive cell killing (Table 4.1). All three HANs demonstrated they were capable of shifting the cell cycle distribution toward high ploidy in concentration-dependent manner. Concentrations of 900 µM CAN, 12 µM BAN, and 6 µM IAN were chosen for subsequent experiments.

4.3.3 Effect of initial cell loading

To examine the effect of cell confluency on the cell cycle modulation, CHO cells were plated at 2×10^5/well/(2 mL F12+5% FBS) or 4×10^5/well/(2 mL F12+5% FBS) in a 6-well tissue culture plate. These cells at different density were treated with HANs in parallel and cell cycle distribution after 26 hour post-

incubation was observed (Figure 4.5). Negative control cells with high cell density have suppressed 2N-4N population (23.9%) compared to the low density cells (53.2%), which indicates the suppression of cell growth (cell cycle arrest) due to cell contact inhibition. IAN- and BAN-treated cells showed distinctly different cell cycle profiles depending on the cell density. When cells were treated with BAN at low cell density, cell population was shifted toward high ploidy (≥4N = 58.7%) after 26 hour post-incubation while BAN treatment at high cell density did not shift the cell population toward high ploidy as much (≥4N = 25.2%). Likewise, IAN treatment at high density resulted in cells with ≥4N content DNA as 39.6% as opposed to 89.5% when cells were treated at low density. Cell cycle alteration by CAN treatment was not affected by cell density; the cell population distribution was 18.2% (2N), 17.7% (2N-4N) and 64.1 % (4N) at low cell density and 20.9% (2N), 21.1% (2N-4N) and 58.0% (4N) at high cell density.

4.3.4 Time-course observation of HAN-treated cells

To determine the time point when HAN treatment exerted cell cycle alteration during post-incubation, HAN-treated cells were collected at 0, 8, 17, 26, 34, 43 and 52 hour post-incubation (Figures 4.6 and 4.7). After 8 hours, IAN-treated cells started to shift toward 4N. After 17 hours, 2N cell population nearly disappeared (2N = 2.55%) and cells started to accumulate at 8N (9.67%). IAN-treated cells accumulated at 8N in direct proportion to incubation time (8.05% and 23.6% at 26 and 34 hours, respectively) but 8N cells decreased to 12.6% at 43 hours followed by 8.11 % at 52 hours of post-incubation. A similar pattern was observed for CAN and BAN-treated cells. Cell accumulation at 8N was not observed for negative control cells. The accumulation of 8N cells coincided with the suppression of cell growth (Figure 4.8). Rather than increasing, IAN-treated cells decreased the cell number from 3.39×10^5 cell/well immediate after the treatment to 2.13×10^5 cell/well at 34 hour post-incubation, followed by an increase to 3.73×10^5 cell/well at 52 hour post-incubation. BAN-treated cells decreased in number for the first 17 hours of post-incubation (3.27×10^5 cell/well to 2.14×10^5 cell/well), and gradually increased thereafter up to 4.27×10^5 cell/well. The number of CAN-treated cells decreased to 1.71×10^5 cell/well at 26 hours of post-

incubation and never exceeded the cell number at 0 hour. Cell viability was over 90% for all treatment groups during the observed post-incubation.

4.3.5 Cell cycle alternation by ethylmethane sulfonate

Although AraC is a potent cell cycle blocker, the mode of action of AraC is the incorporation into DNA strand elongation, which does not happen with HAN. HANs are known to form DNA adducts [20] and these adducts may contribute to cell cycle alterations by HANs. Ethylmethane sulfonate (EMS) is a well-known monofunctional alkylating agent that binds to nucleophilic centers of DNA at the O^6 and N^7 positions of guanine, N^3-adenine and O^2-thymine with the ratio of *N*-versus *O*-alkylation of ~30 [52, 53], and it is widely used as a model compound [54]. EMS-treated cells shifted the cell distribution toward 4N at 26 hour post-incubation (41.0% for EMS-treated cells as opposed to 20.2% for negative control cells), and the (2N-4N) + 4N population was higher at 52 hour post-incubation (74.2% for EMS-treated cells as opposed to 47.5% for negative control cells) (Figure 4.9). However, EMS-treated cells did not express a large percentage of 8N cells.

4.3.6 Cell visualization

Microscopic investigation demonstrated normal spindle-shape cell morphology for the negative control cells (Figure 4.10). Treatment for 4 hours with HANs changed the cell morphology to round shape. After 26 hours of incubation, HAN-treated cells still looked mostly round rather than normal elongated spindle shape. After another 26 hours, HAN-treated cells showed spindle shape, but the size of the spindle looked larger compared to negative control cells. The difference of cell size is more prominent with the imaging of harvested cells (Figure 4.11). Harvested cells are spherical because they were isolated from the plate wells using trypsin. Negative control cells exhibited similar cell size at 0, 26 and 52 hour post-incubation, but HAN-treated cells at 26 and 52 hour post-incubation were larger and more variable compared to negative control cells. Treatment with 10 mM EMS and 50 μM AraC did not change the

morphology of CHO cells right after the 4-hour treatment, but these agents also made cells larger at 26 and 52 hour post-incubation.

4.3.7 Plating efficiency

The ability of cells to proliferate after exposure to HANs was examined to determine the association of cell growth suppression with cell cycle alteration. After treatment for 4 hours, cells were washed and collected. Equal numbers of treated cells were plated onto a new 96-well plate and the resulting cell densities after 72 hours were compared to their concurrent negative controls. The concurrent negative controls had their mean density set to 100%. HAN-treated cell growth was suppressed and at the concentrations used for cell cycle analysis, the mean (±SE: standard error of the mean) cell densities compared to their concurrent negative controls were 14.0 ± 0.8%, 28.9 ± 1.8%, and 27.2 ± 1.8% for 900 µM CAN, 12 µM BAN, and 6 µM IAN, respectively. Cells treated with10 mM EMS or 100 µM AraC also had suppressed cell growth with mean cell density of at 34.9 ± 0.7% and 46.8 ± 1.2%, respectively, compared to their concurrent negative controls (Figure 4.12).

4.3.8 DNA strand breaks

Alkaline single cell gel electrophoresis detects incompletely repaired DNA damage, as alkali labile sites, as well as DNA single and double strand breaks. CHO cells were collected immediately after the chemical treatment as well as after 26 hours post-incubation, embedded in agarose on a microscope slide and lysed prior to examining the electrophoretic migration of genomic DNA. Treatment with 900 µM CAN resulted in extensive DNA migration (% tail DNA = 60.7), which reduced to % tail DNA of 7.72 after 26 hours post-incubation. BAN at 12 µM induced moderate DNA migration (% tail DNA = 23.2) and reduced to negative control level (% tail DNA = 0.11). IAN at 6 µM induced a weak level of strand breaks resulting % tail DNA of 2.21 and became 0.01 after 26 hour post-incubation (Figure 4.13).

4.4 Discussion

Eukaryotic cells have multiple checkpoints to ensure the integrity of the genome and cell division [38, 55, 56]. DNA integrity checkpoints monitor DNA status and prevent inappropriate transitions into S phase and mitosis when aberrations are detected. DNA damage causes cell cycle delay before S phase, during replication and before mitosis [57]. Proliferating cells that traverse through G_1 or G_2 phases can respond to genotoxic stress by activating checkpoints that impose shorter or durable cell-cycle arrests in G_1 or G_2, before re-entry into S phase or mitosis [58]. Cells that experience genotoxic stress during DNA replication only delay their progression through S phase in a transient manner, and if damage is not repaired during this delay they exit S phase and arrest later when reaching G_2 checkpoint. The replication checkpoint, the S-M checkpoint, and the intra-S-phase checkpoint do not requires p53, which is the key target of the sustained G_1 (G_1-S) checkpoint that arrests the cell cycle in G_1 phase [59]. A subject review indicated that the checkpoint may contribute to the generation of mutations rather than their prevention through translesion DNA synthesis regulation [60].

Eukaryotic organisms usually contain a diploid complement of chromosomes. The diploid state (2N) enables sexual reproduction and facilitates genetic recombination. Although some plants and fungi can deviate from diploid, the normal state of the animal cell is diploid. Duplication of mammalian nuclear DNA is restricted to only once each time the cell divides. However, the chromosome complement can differ within an organism by cell type, and in many cancer cells the number and the structure of each chromosome can be highly variable [61, 62]. HANs are known to induce DNA damage detectable by SCGE assay [8] with the descending rank order of IAN > BAN > CAN (Figure 4.1). Thus the association of genotoxic damage with cell cycle arrest is of interest.

This is the first research study that has identified cell cycle alteration by a class of DBPs. It was shown in this study that HAN treatment shifted the cell cycle distribution toward 4N after 26 hours of post-incubation (Figure 4.4). Interestingly, DNA strand breaks induced by HANs were mostly repaired after 26 hours (Figure 4.13). At first this cell cycle shift was interpreted as G_2 cycle arrest, but extending the post-incubation up to 52 hours as well as time-course analysis revealed the presence of an

accumulating 8N cell population (Figure 4.6). It appeared that 4N cells further went through another cell cycle. Accumulation of 8N cells was most pronounced for IAN-treated cells at 34 hours of post-incubation (23.6%). The accumulation of 4N and 8N cell population may reflect the inhibitory effect of HANs into the mitotic entry. Alternatively, cell proliferation may proceed without progression to mitosis. IAN treatment almost eliminated 2N cell population (2.55%) as early as 17 hours of the post-incubation (Figure 4.6). AraC is a strong positive control but it only affects the DNA strand elongation in S phase. The fact that not all the cells were affected by AraC can be seen in the microscopic imaging of AraC-treated cells after 52 hours of post-incubation (Figure 4.10). There are cells with equivalent sizes with negative control cells as well as much larger cells. CHO cells were not synchronized at the time of chemical treatment and that explains why only a fraction of the cells was affected by AraC treatment. The fact that the 2N cell population in IAN-treated cells was eliminated at 17 hours post-incubation indicates that IAN can act on cells at any point of cell cycle and affect high ploidy.

HANs have two reactive centers: (a) displacement of a halogen atom at α-carbon by S_{N2} reaction, and (b) addition at the partially positively charged carbon of cyano groups [63] (Figure 4.14). BAN and CAN were shown to react with calf thymus DNA at guanine base to form a 7-(cyanomethyl)guanine DNA adduct [20]. The rank order of cell cycle alteration (Figure 4.4) and the rank order of DNA damage induction (Figure 4.1) follows the same order (IAN > BAN > CAN), which may signal a mechanism that involves the α-carbon. HAN treatment was thus compared to EMS, which is a well-established alkylating agent that forms (mostly ethyl) adducts at the *N*- and *O*-atoms in DNA bases [52, 53]. Alkyl DNA adducts exhibit different stabilities. In general, *O*-alkylations are highly mutagenic and genotoxic, whereas *N*-alkylations are cytotoxic but less mutagenic [54, 64]. EMS at a concentration of 10 mM induced substantial amount of DNA strand breaks in CHO cells after 4 hour treatment [65]. In this study EMS-treated cells shifted the cell cycle distribution toward 2N-4N and 4N DNA content compared to concurrent negative control but did not proceed to 8N state (Figure 4.9). Treatment of CHO cells with 10 mM EMS suppressed the cell growth (Figure 4.12) and resulted in larger cells after 26 and 52 hours of

post-incubation (Figure 4.10). Lack of accumulation of 8N cells indicates that direct alkylation of DNA cannot account for the cytogenetic toxicity of the HANs.

Cytometric analysis only quantifies dye-incorporation to examine DNA content and does not reflect the biological complexity of the cell cycle. Sakaue-Sawano and colleagues reported that an inhibitor of DNA topoisomerase II halted HeLa (immortal cancer cell line derived from human adenocarcinoma) and normal murine mammary gland (NMuMG) cells at the G_2/M checkpoint but NMuMG cells then overrode the checkpoint and underwent nuclear mis-segregation or avoided the checkpoint and entered the endoreplication cycle, in which cells increased their genomic DNA content without dividing [41]. Cell cycle progression is dependent upon the coordinated regulation of ubiquitination, and they harnessed this system to develop a genetically encoded fluorescence indicator of cell cycle progression (Fucci: *F*luorescent *U*biquitination-based *c*ell *c*ycle *i*ndicator). NMuMG/Fucci2 cell nuclei progressed from yellowish green (indication of S/G_2/M) to red (indication of G_1) without undergoing cell division. No cellular features consistent with M phase were seen, including nuclear envelope breakdown and chromosome condensation, during the yellowish green to red color conversion. Increases in cellular DNA content suggested a transition from the mitotic to endoreplication cycles by bypassing mitosis and cytokinesis (separation of cytoplasm of daughter cells). NMuMG/Fucci2 cells underwent only one round of DNA endoreplication generating large red nuclei, confirming cells remained tetraploid for an extended period up to 96 hours in the presence of 10 µM etoposide (topoisomerase II inhibitor; topoisomerase II segregates chromosomes at cell division). A small fraction of the NMuMG cells was observed to go through multiple endoreplication cycles in the presence of 1 – 3 µM etoposide. Time-lapse high-resolution imaging analysis at the single cell level combined with population analysis allowed Sakaue-Sawano et al. [41] to explore the processes of cell cycle alteration in great detail. Conventional flow cytometric cell cycle analysis that only quantifies dye-incorporation may have misinterpreted the long-lasting tetraploid state as G_2 arrest. Flow cytometric analysis in this study only measured propidium iodide incorporation into DNA and thus cannot distinguish if 4N cells are diploid at G_2/M or tetraploid

cells at G_1. That is why the cell cycle distribution is displayed as 2N, 2N-4N, 4N and 4N-8N and 8N in Figures 4.3 – 4.7, and 4.9.

Polyploid cells in a diploid organism can form by three general mechanisms: 1) cell fusion, 2) a developmental process known as endoreplication, and 3) a variety of defects that result in an abortive cell cycle. Endoreplication is an example that polyploidy of cells within an organism can be programmed as a feature of normal development and can be seen in *Drosophila* salivary glands or mammalian megakaryocytes. During this specialized cell cycle, cells skip mitosis and proceed through several rounds of DNA replication, resulting in autopolyploid cells (multiples of a single genome), which generally do not proliferate further. Abortive cell cycles arise from a variety of defects in different aspects of cell division: DNA replication, dissolving sister-chromatid cohesion, mitotis spindle function and cytokinesis. Many of the defects that result in abortive cell cycles trigger checkpoint responses that block cell-cycle progression or, in some cases, trigger apoptosis [61].

Various checkpoints exist at the mitosis to ensure the fidelity of cell replication, alignment of all chromosomes at methaphase, formation of mitotic spindle, and interaction of kinetochores with spindle microtubules. Andreassen and colleagues treated mitotic CHO cells with chemical agents that inhibit different stages of mitosis (disruption of either the function of mitotic checkpoints or the coordination of mitotic event) and all chemicals forced CHO cells out of mitosis without undergoing cell division. After two additional cell cycles tetraploid cells remained viable and rapidly became aneuploid [66].

When animal cells become tetraploid, they acquire extra centrosomes that could potentially compromise the assembly of a bipolar spindle. Centrosome is a structure that serves as the main microtubule-organizing center in animal cells and define the spatial organization of the microtubule cytoskeleton [67]. Multipolar mitosis leads to aneuploidy [61]. Aneuploidy (state of abnormal chromosome number and content) is the most commonly observed genetic alteration in many types of tumors [62]. Populations of tetraploid cells are found in a variety of human tumors where they may act as precursors of aneuploidy and tumorigenesis [68, 69]. Animal cells have self-defense system to eliminate tetraploid cells, called “tetraploidy checkpoint”. Tetraploidy checkpoint enhances the fidelity of cell

proliferation by preventing aneuploidization by inducing the tetraploid cells to withdraw from the cell cycle before DNA replication [70, 71].

Andreassen and colleagues showed that tetraploid state itself act as a checkpoint to arrest cells in G_1 regardless of the induction mechanism of tetraploidy [71]. Nontransformed primary rat embryo fibroblasts (REF)-52 cells went indefinitely into tetraploid G_1 arrest upon drug-induced mitotic failure. G_1 arrest involved inactive Cdk2 kinase (required for G_1-S progression), hypophosphorylated retinoblastoma protein (pRB) (a gatekeeper of the pathway of activation of S phase and entry into a new cell cycle), and elevated levels of cell cycle progression regulating protein $p21^{WAF1}$, and cyclin E. Similar tetraploid G_1 arrest was obtained with nontransformed mouse NIH3T3 and human IMR-90 cells. On the other hand, transformed derivative of REF-52 cells with inactivated p53 (p53DD cell) did not arrest but accumulated with 8N DNA content with mitotic activity. After release from the drug treatment, p53DD cells proceeded through a full cell cycle and became highly aneuploid with DNA content ranging from < 2N to 4N.

Arrest in the G_1 phase of the cell cycle is mainly mediated by the p53 tumor suppressor, a transcription factor that induces cell cycle arrest, damage repair, and apoptosis [72]. The cell cycle effects of p53 activation include induction of transcription of Cdk inhibitor p21. The induction of p21 in response to DNA damage is the major mechanisms enforcing arrest in G_1 in response to DNA damage [58]. Drug-induced mitosis exit induced indefinite G_1 arrest in nontransformed REF-52 cells [71] but not in CHO cells [66]. CHO cells that became tetraploid following exposure to 2-aminopurine (protein kinase inhibitor), taxol (microtubule assembly inhibitor), ICRF-193 (topoisomerase II-dependent DNA decatenation inhibitor). or dihydrocytochalasin B (actin assembly inhibitor) all became aneuploid within 30 hours, which represents two subsequent cell divisions after release from treatment.

The parental cell line of CHO AS52 is CHO-K1 [44]. CHO-K1 contains a single missense mutation at position 633 (codon 211). This base difference corresponds to an amino acid change from threonine (ACA) to lysine (AAA) in the DNA-binding domain and CHO-K1 lacks the ability to induce G_1 checkpoint [73-75]. Hu et al. identified the p53 status in CHO-K1 as mutant protein due to its high spontaneous levels that are non-inducible after X-irradiation [73]. On the other hand, Tzang et al.

concluded that the mutation does not have effect on the functional properties of the p53 protein and the lack of G_1 arrest of CHO-K1 is likely p53-independent; rather, Tzang attributed the inability of CHO-K1 for G_1 arrest to deficiency of p21 (Waf1/Cip1) expression since transfecting the CHO-K1 cells with a wild-type human p53 gene failed to retrieve the ability of G_1 arrest upon X-ray irradiation [74]. However, it is reported that sequence-specific binding function is lost in point-mutated p53 proteins and many of the mutant p53 can act as dominant negative to inhibit this activity of the wild-type p53 [72, 76]. Combined with the report from Hu et al. [73], it is most likely that the function of p53 in CHO-K1 is somewhat compromised.

Since the CHO AS2 cell used in this study is a derivative of CHO-K1 cell, it is most likely that the gene encoding p53 in AS52 carries the same mutation as in K1 cell line. HAN-treated CHO AS52 cells showed accumulation of cells with > 4N DNA content after 17 hours of post-incubation. The high ploidy cells accumulated with time but the proportion of high ploidy cells went down after 34 hours of post-incubation. It is not likely that 8N cells could segregate normally to become diploid. Polyploid cells cannot overcome the issue of multipolar mitosis, which leads to aneuploidy [61]. When synchronized mitotic cells were treated with mitosis inhibitor, resulting aneuploidy cell cycle distribution was wide spread of DNA content ranging from 2N to more than 8N without distinct peaks [66, 71]. The “noisy” cell cycle profile of IAN-treated cells at 34, 43, and 52 hours may be an indication of aneuploidy cells (Figure 4.6). A small cell population can be observed in subG_1 region of BAN-treated cells at 26, 34 and 43 hours. Cell cycle profile of CAN-treated cells did not appear as sharp as the concurrent negative control cells, but not as diffuse as the IAN-treated cells. It may be questioned why distinct peaks are observed in the cell cycle profiles of HAN-cells instead of broad distribution as was seen in [71], after accumulation of 8N cells. An explanation may be that cells are not synchronized at the treatment and that HANs acted on cells at a specific cell cycle stage. It may be argued that an aneuploid cell population may have been lost at sample collection since cells at designated post-incubation were rinsed with HBSS first and subsequently trypsinized. Floating cells were observed by visual inspection but accurate quantification of detached cells was not practically possible. A preliminary experiment examined the hypothesis that

collecting only adherent cells would not bias the cell cycle population. Parallel wells were prepared for CAN at 900 µM, BAN at 12 µM, IAN at 6 µM and EMS at 10 mM. Cells were collected right after the 4-hour treatment (= 0 hour), and 26 and 52 hour post-incubation. One well was rinsed with HBSS first and trypsinized to collect only adherent cells. From the other well, rinsing solution and harvested cell suspension were collected to count whole cells (floating cells + adherent cells). There were no significant differences in cell cycle profile between whole cells and adherent cells in any treatment groups (data not shown) thus the hypothesis was accepted, and only adherent cells were collected hereafter.

The cell number of IAN- and BAN-treated cells started to increase after 34 hours. Increase of the cell number may imply that there was an "unaffected cell population" retaining their normal ploidy, and that this unaffected population may have corresponded to distinct peaks in the cell cycle profiles at later post-incubation times. Nevertheless, it is difficult to conclude the fate of cells with different DNA content without a real-time observation at a single cell level.

Andreassen et al. successfully induced tetraploid CHO cells by override of cell cycle chekpoints, inhibition of microtubule assembly, inhibition of topoisomerase II and inhibition of cleavage furrow formation [66]. In order for a cell to be a tetraploid, it has to attempt mitosis. Progression of cell cycle may be necessary for HANs to exert their toxicity. When initial CHO cell loading was 4×10^5 cells/well, cell cycle alteration by BAN and IAN was not as prominent as for cells with the initial loading of 2×10^5 cells/well (Figure 4.5). With the higher cell density it is very likely more cells exerted cell contact inhibition during the 4-hour treatment and/or early in the post-incubation, thus the progression of cell cycle was suppressed. There might be less number of cells that attempted mitosis with high cell density.

The HAN treatment condition was first chosen from the DNA repair kinetics work (Chapter 3). The respective concentrations of 60 µM, 60 µM, and 1200 µM for IAN, BAN, and CAN turned out to be too high for the ploidy analysis. Almost all cells were dead after 26 hours of post-incubation with IAN at 60 µM and BAN at 60 µM, and cells treated with CAN at 1200 µM were half detached from the surface of the 6-well plate. Initial cell density for DNA repair kinetics experiment was 4×10^4 cells/0.32 cm^2/96-well (= 1.25×10^5 cells/cm^2) whereas initial cell density for cell cycle analysis was 2×10^5 cells/9.5

cm^2/6-well (= 2.11×10^4 cells/cm^2). Moreover, during the post-incubation, serum-free medium was used for DNA repair kinetics and complete medium was used for cell cycle analysis; cell cycle progression was doubly suppressed by cell confluence status and serum starvation for the repair experiment. HAN concentrations were lowered to 6 µM, 12 µM, and 900 µM for IAN, BAN, and CAN, respectively, for the cell cycle analysis. The same trend was observed for the HAN-treated cell plating efficiency. The concentration range for plating efficiency in this work is lower (2 – 8 µM for IAN, 2 – 14 µM for BAN, and 200 – 1200 µM for CAN) when cell treatment was at low cell density in this work (Figure 4.12) compared to the higher concentration range for plating efficiency at confluence (45 – 60 µM for IAN, 43 – 60 µM for BAN, and 460 – 1200 µM for CAN, Chapter 3). It was also demonstrated that contact-inhibited mouse cells displayed a very strong suppression of UV-C-induced apoptosis when they were treated in a confluent state, which supported their conclusion of proliferation dependence of the induction of apoptosis by UV-C light [42].

In conclusion, it appears HAN could induce tetraploid cells by inducing mitotic failure in CHO AS52 cells. Due to the lack of G_1 arrest of CHO cells, HAN-treated cells underwent another cell cycle and became tetraploid. Further progression of cell cycle resulted in induction of high ploidy cells (> 4N). Tetraploid cells can be induced by override of cell cycle checkpoints, inhibitions of microtubule assembly, inhibition of topoisomerase II and inhibition of cleavage furrow formation [66], some of which could be a mechanism of toxic action of HANs. In p53-competent cells, tetraploid checkpoint triggers apoptosis to eliminate tetraploid cells [71]. The lack of subG_1 population may confirm the lack of p53-dependent apoptosis pathway in CHO AS52 cells.

Mutations of the p53 tumor suppressor gene are common in various types of human cancer [77]. Alterations in the p53 tumor suppressor gene appear to be important in the development of many human tumors. The wild-type p53 gene has a polymorphism at codon 72 that presents the arginine (CGC) or proline (CCC) genotype, which has been associated with increased risk or urothelial cancer in smokers [78]. Thus induction of aneuploidy by HANs may be a threat to vulnerable subpopulations.

There are accumulating evidences of transient 4N state proceeding aneuploidy in cancer progression [39, 69]. Aneuploidy is a hallmark of tumorigenesis [39]. An increase in ploidy generally affects genome instability [61]. Polyploid fission yeast undergoes chromosome missegragation at a high frequency. Tetraploid budding yeast cells showed increased sensitivity to gamma irradiation and to other DNA-damaging agents. If it is also true for mammalian cells, HANs-exposed cells may become susceptible to other DNA-damaging agents.

Epidemiological studies have associated DBPs with urinary bladder cancer but rodent single-chemical bioassays have not been able to demonstrate tumor induction at the same organ sites [1, 79]. There are no mechanistic data on how regulated organic DBPs can contribute to bladder cancer. We have yet to identify a plausible causal agent for adverse human health outcomes. Induction of aneuploidy in HAN-treated cells may imply HANs as a player in cancer induction and adverse pregnancy outcomes associated with long-term exposure to disinfected drinking water. Due to increasing stress on source water availability, many utilities seek alternative disinfection processes or alterations to chlorination practices. Regulated DBPs are used as general indicators for DBP control but many scientists believe that regulated DBPs do not account for the adverse health outcomes identified by epidemiology studies. Identification of major DBP components associated with adverse health outcomes from disinfected drinking water will aid in developing science-based DBP control strategies for drinking water treatment.

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Tables and Figures

Table 4.1 Trypan blue cytotoxicity of HAN-treated cells at incremental concentrations. After 26 hours of post-incubation.

CAN	Viability (%)	BAN	Viability (%)	IAN	Viability (%)
0 µM	99.5	0 µM	100.0	0 µM	100.0
700 µM	95.8	9 µM	98.9	2 µM	100.0
750 µM	99.3	10 µM	97.9	3 µM	96.0
800 µM	96.9	11 µM	99.0	4 µM	98.0
850 µM	95.8	12 µM	98.2	6 µM	94.3
900 µM	95.3	13 µM	95.9	8 µM	98.9

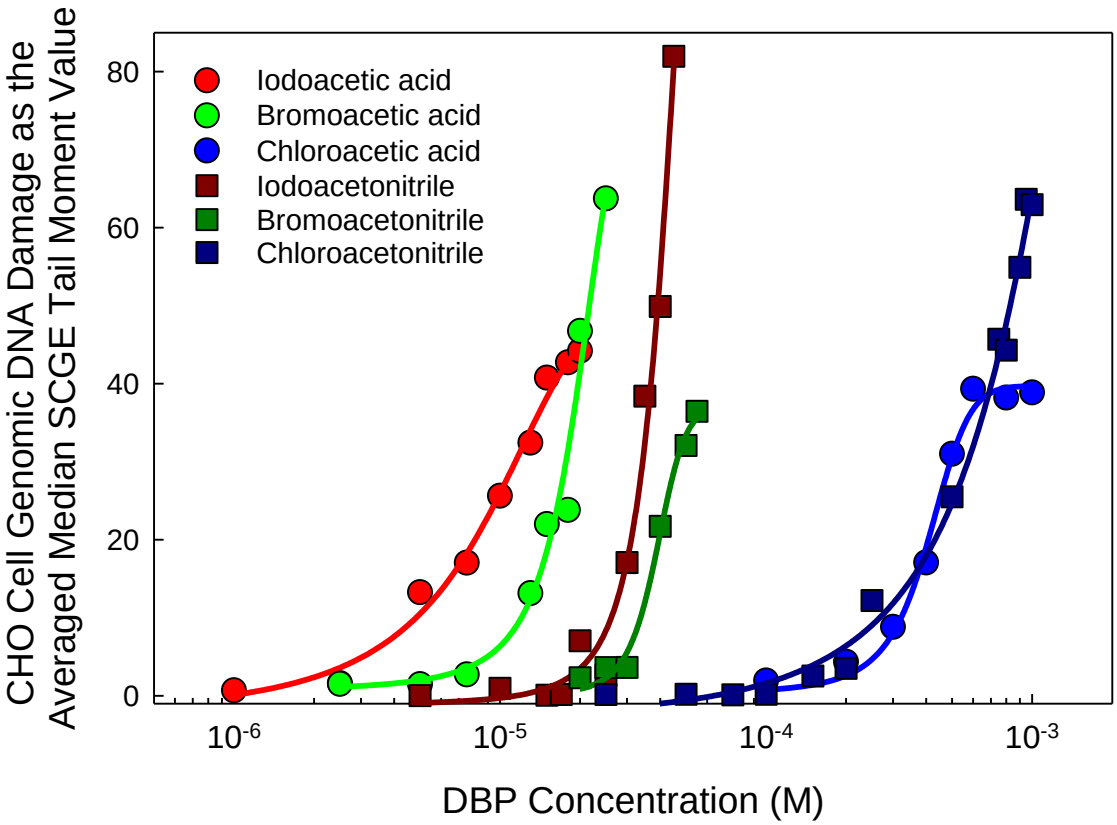

Figure 4.1 Genomic DNA damage in CHO cells by monohaloacetic acids and monohaloacetonitriles (data from [8, 33]).

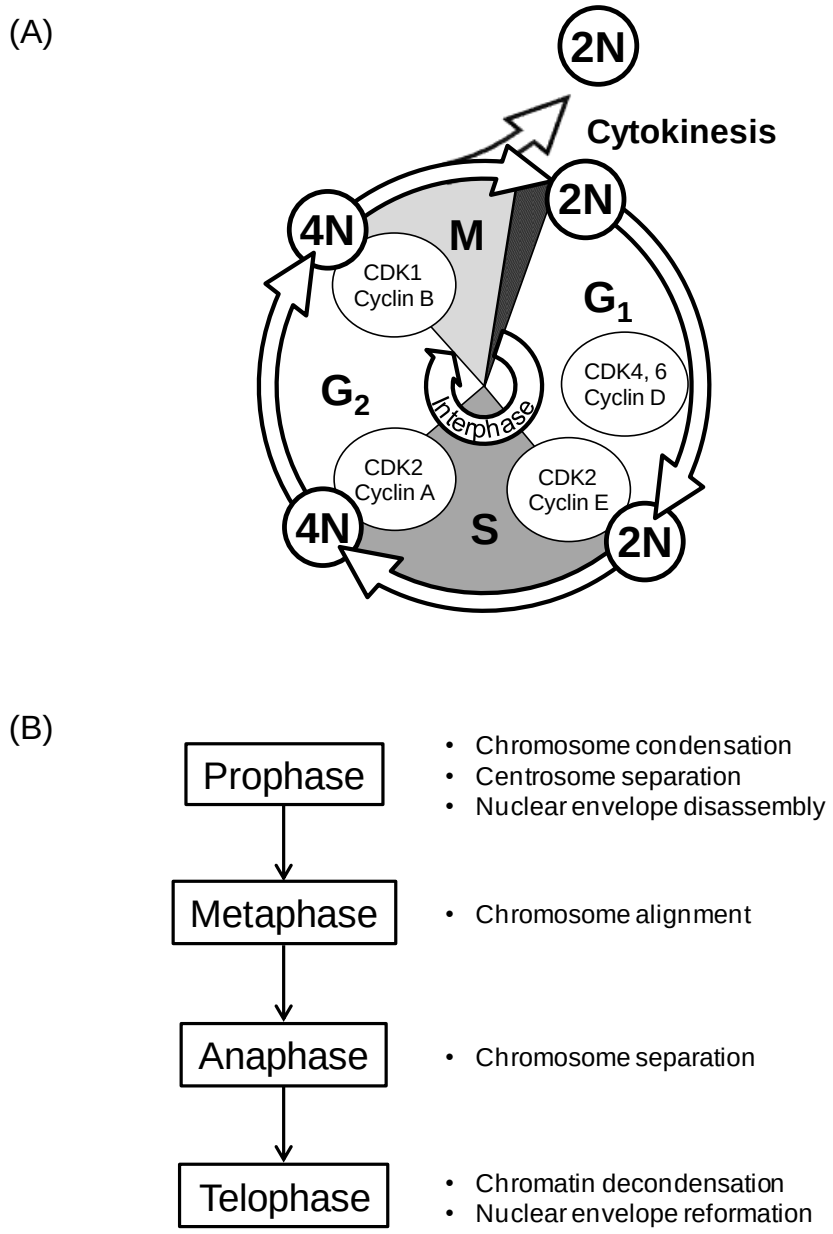

Figure 4.2 (A) Schematic outline of eukaryotic cell cycle. (B) Phases of cell mitosis (M phase).

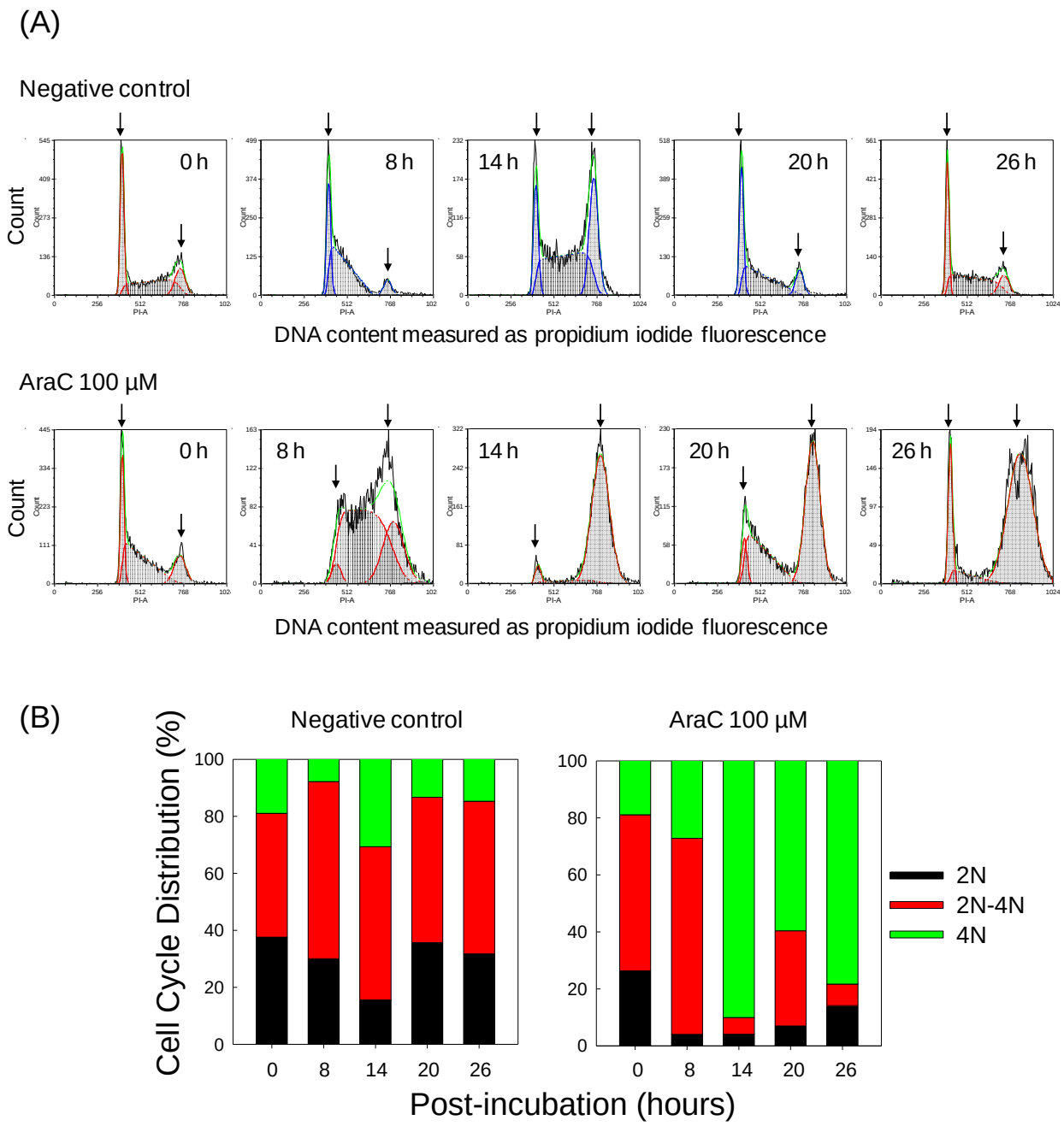

Figure 4.3 Cell cycle profiles of CHO cells treated without or with 100 µM of AraC at various time points during post-incubation. (A) Cell cycle profiles obtained with LSR II flow cytometer. CHO cells were treated with 0 µM or 100 µM of AraC (top and lower panel, respectively) for 4 hours and harvested at various time points during post-incubation. Arrows indicate the 2N and 4N DNA containing cells from the left. (B) The cell cycle distributions were calculated using FCS Express software.

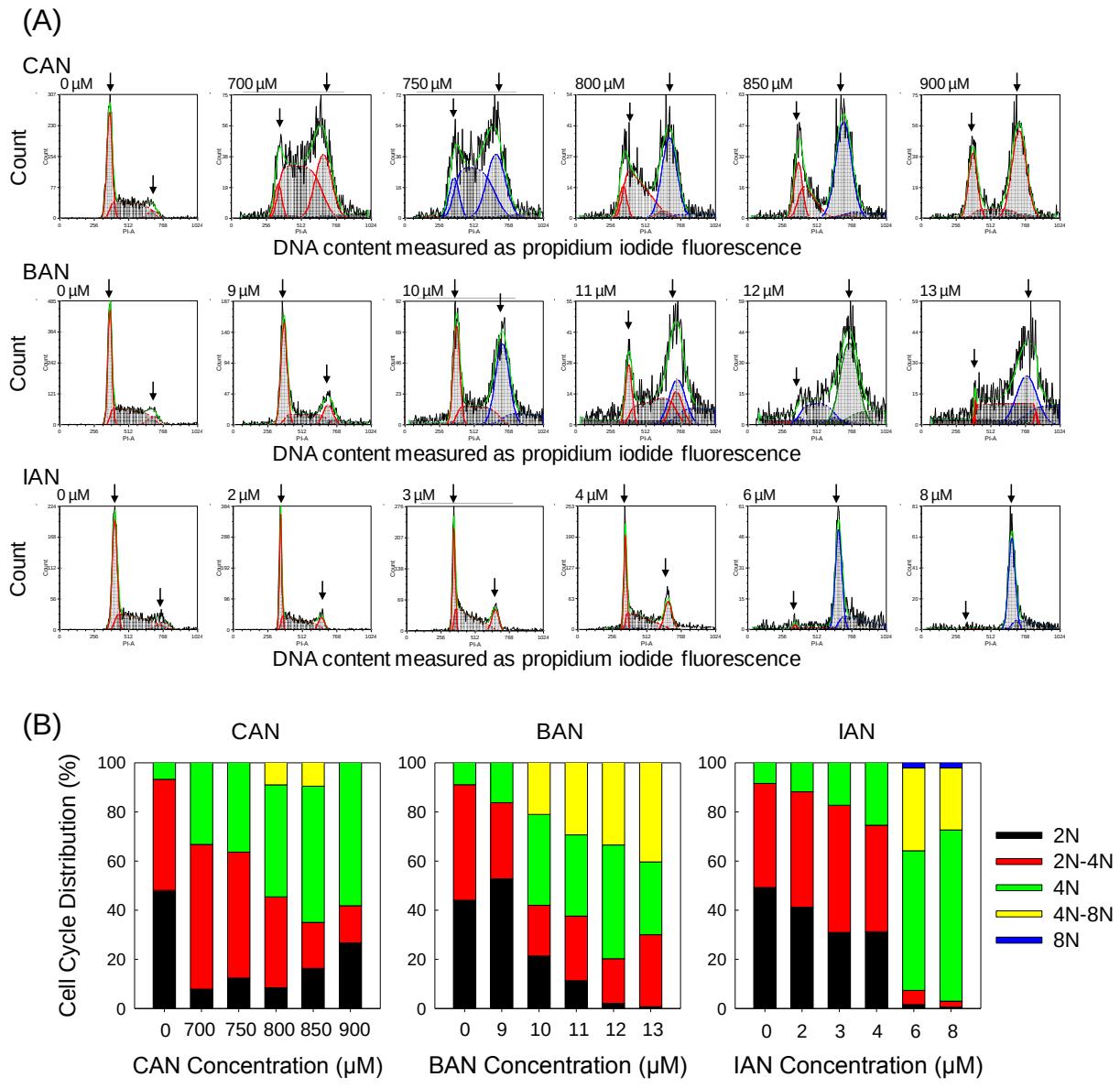

Figure 4.4 Cell cycle profiles of CHO cells treated with various concentrations of HANs at 26 hour post-incubation. (A) Flow cytometric diagram obtained with LSR II flow cytometer. CAN (top), BAN (middle), and IAN (bottom) at listed concentrations. Arrows indicate the 2N and 4N DNA containing cells from the left. (B) The cell cycle distributions were calculated using the FCS Express software. CAN (left), BAN (center), IAN (right).

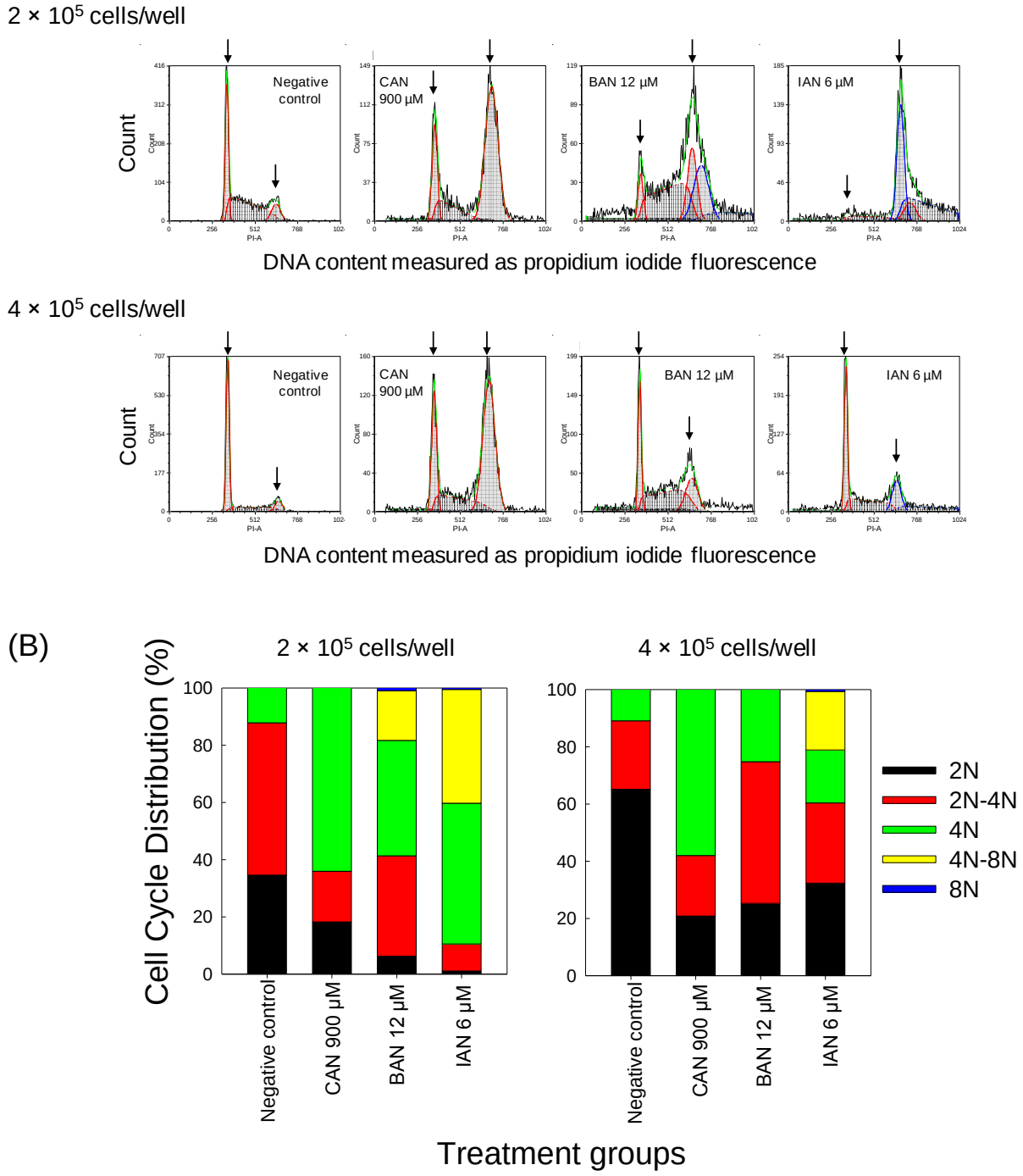

Figure 4.5 Cell cycle profiles of CHO cells treated at different initial cell loading. (A) Flow cytometric diagram obtained with LSR II flow cytometer. Initial cell loading of 2×10^5 cells/well (top), and 4×10^5 cells/well (bottom). Arrows indicate the 2N and 4N DNA containing cells from the left. (B) The cell cycle distributions were calculated using the FCS Express software. 2×10^5 cells/well (left), 4×10^5 cells/well (right).

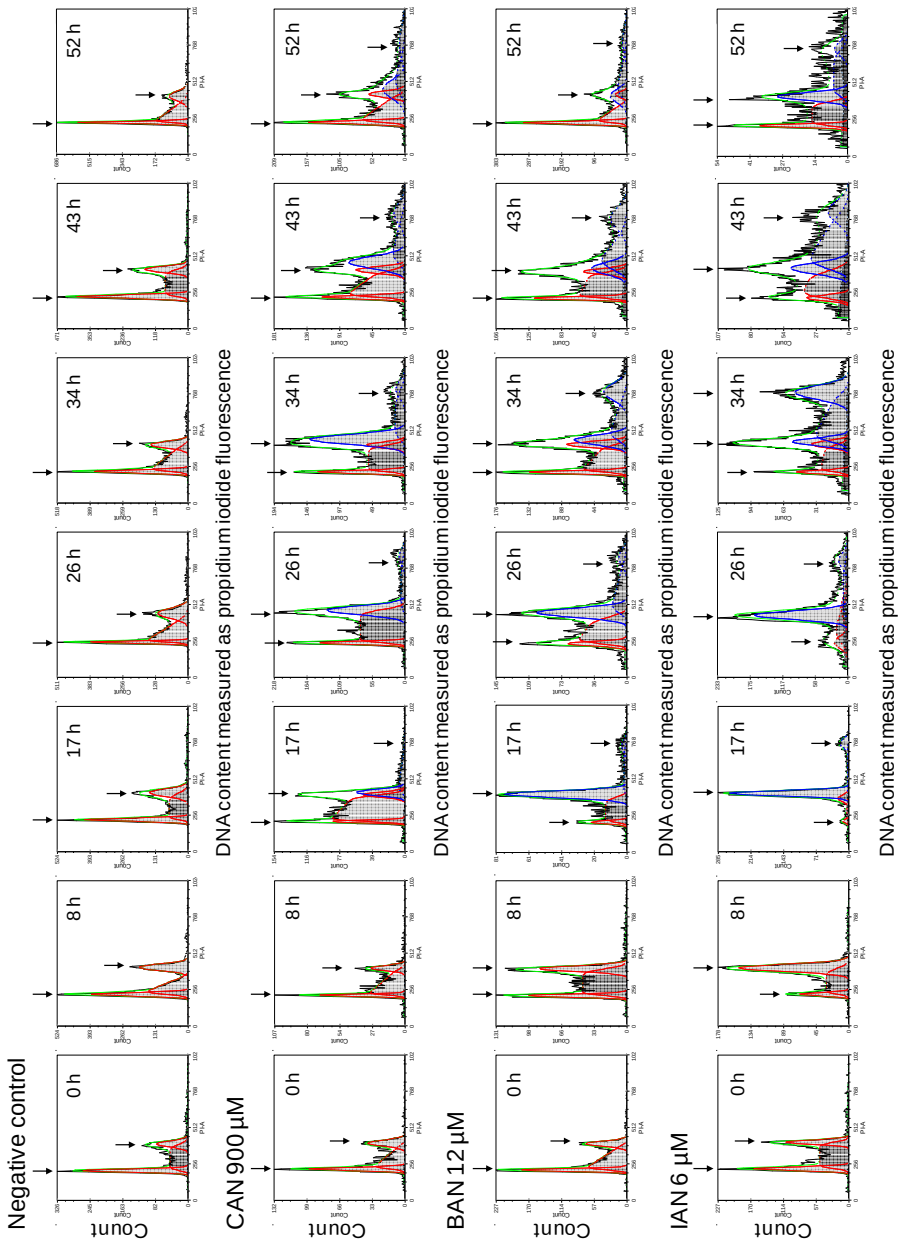

Figure 4.6 Cell cycle profiles of CHO cells at 0, 8, 17, 26, 34, 43, and 52 hour post-incubation. Treatment conditions are negative control, 900 µM CAN, 12 µM BAN, and 6 µM IAN. Cell cycle profiles were obtained with LSR II flow cytometer. Arrows indicate the 2N, 4N and 8N DNA containing cells from the left.

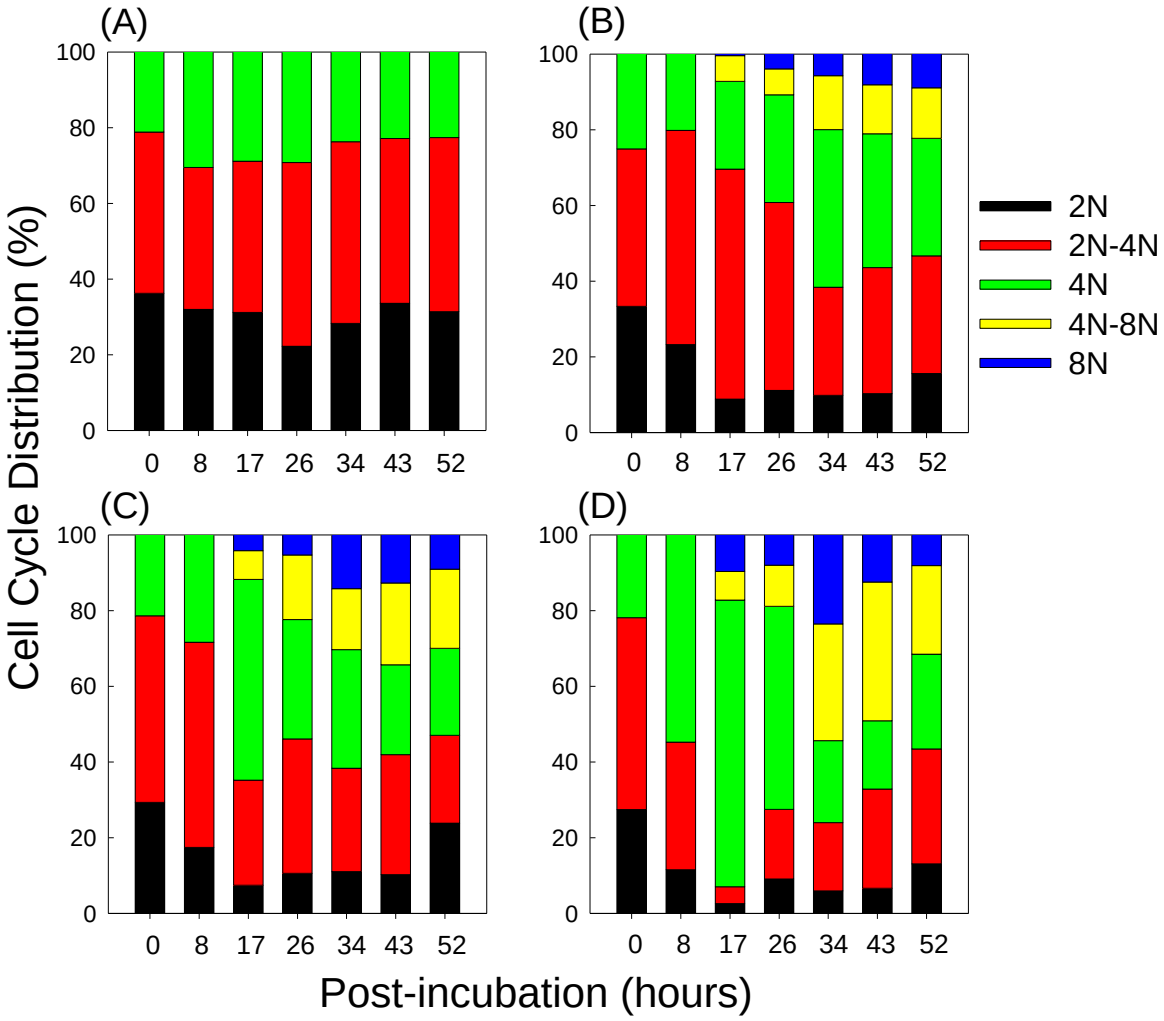

Figure 4.7 CHO cell cycle distributions at 0, 8, 17, 26, 34, 43, and 52 hour post-incubation. The cell cycle distribution was calculated using the FCS Express software. (A) Negative control, (B) CAN at 900 µM, (C) BAN at 12 µM and (D) IAN at 6 µM.

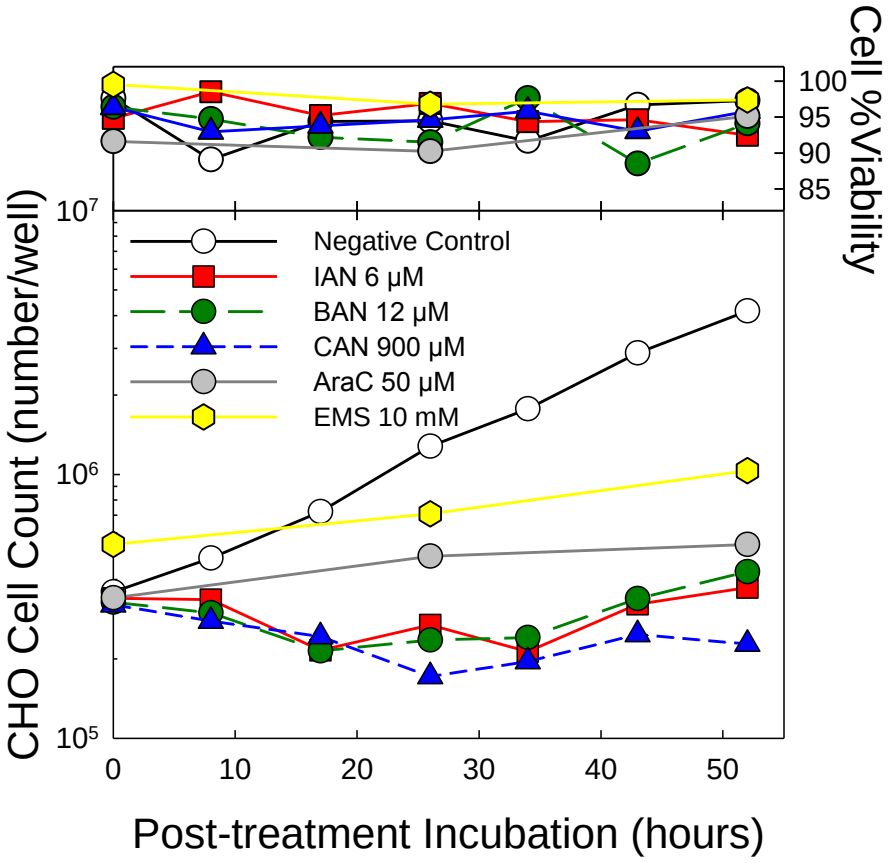

Figure 4.8 CHO cells viability (top) and growth (bottom) curves during post-incubation after treated without or with 900 μM CAN, 12 μM BAN, 6 μM IAN, 50 μM AraC, or 10 mM EMS.

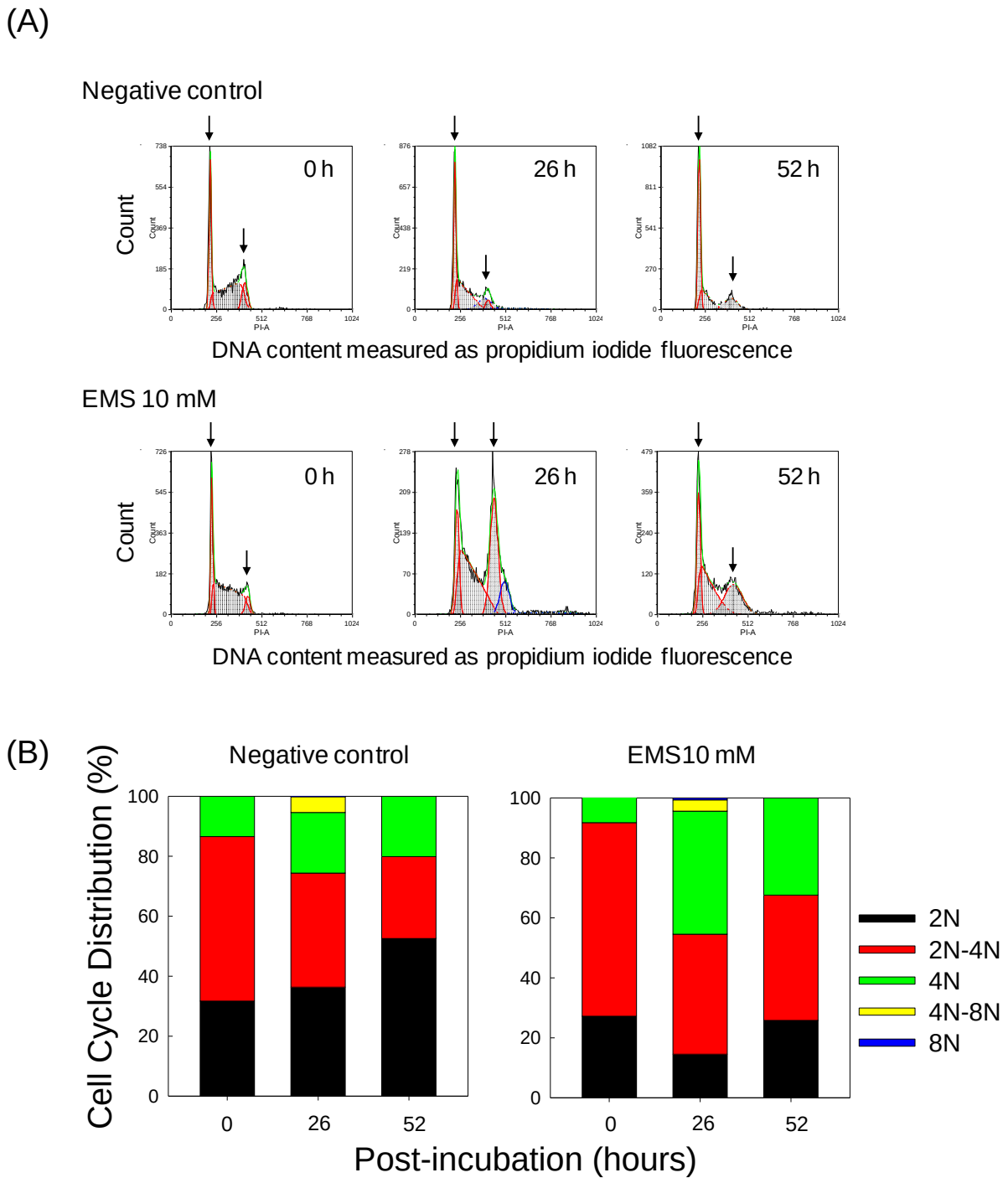

Figure 4.9 Cell cycle profiles of CHO cells at various points during post-incubation. (A) Cell cycle profiles obtained with LSR II flow cytometer. CHO cells were treated without or with 10 mM EMS for 4 hours and harvested at 0, 26, and 52 hour post-incubation. Arrows indicate the 2N and 4N DNA containing cells from the left. (B) The cell cycle distribution calculated with the FCS Express software. Negative control (left), EMS at 10 mM (right).

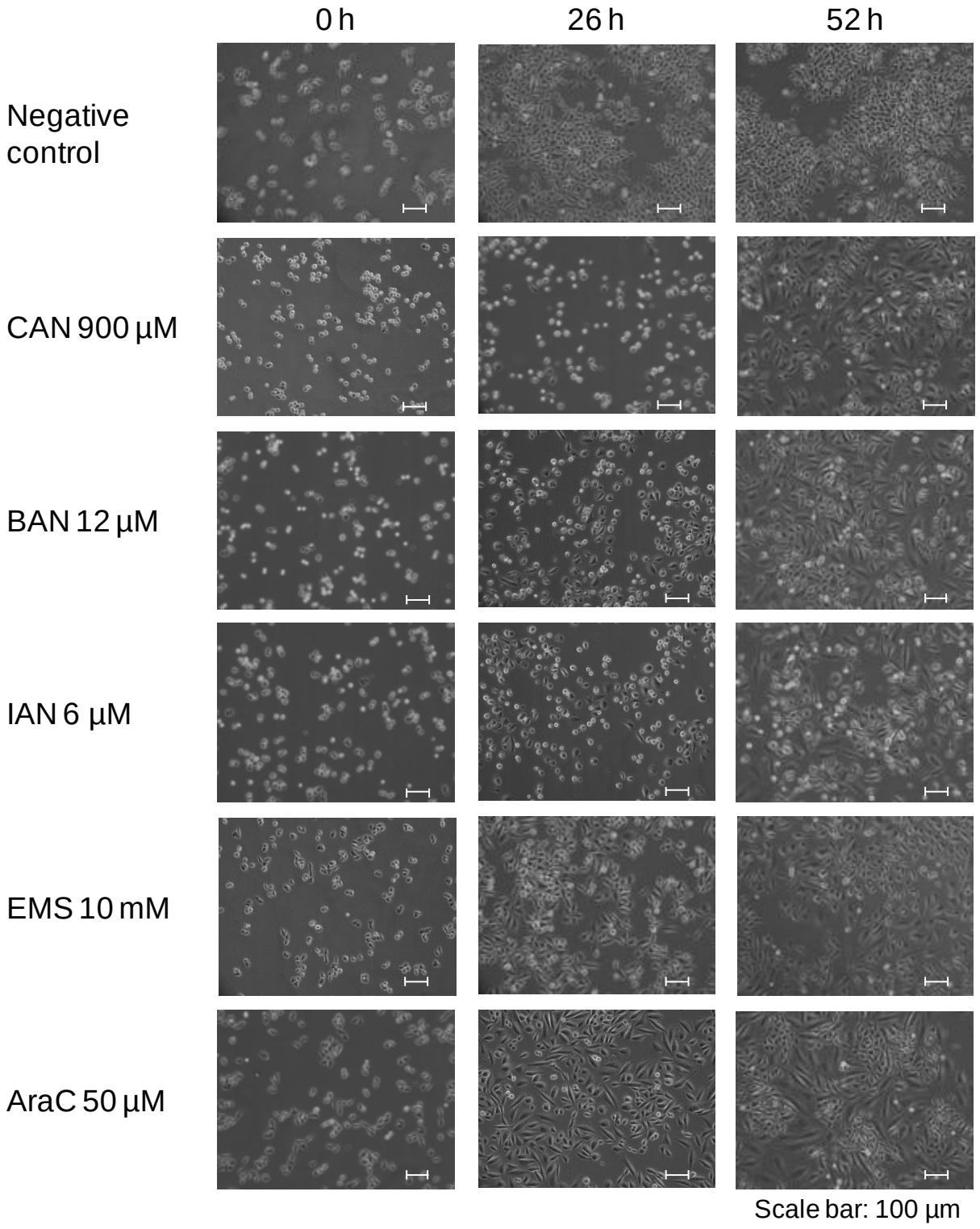

Figure 4.10 Microscopic images of adherent cells with 10× phase contrast objective. CHO cells were observed at 0, 26, and 52 hour post-incubation. Treatment conditions are negative control, 900 µM CAN, 12 µM BAN, 6 µM IAN, 10 mM EMS or 50 µM AraC.

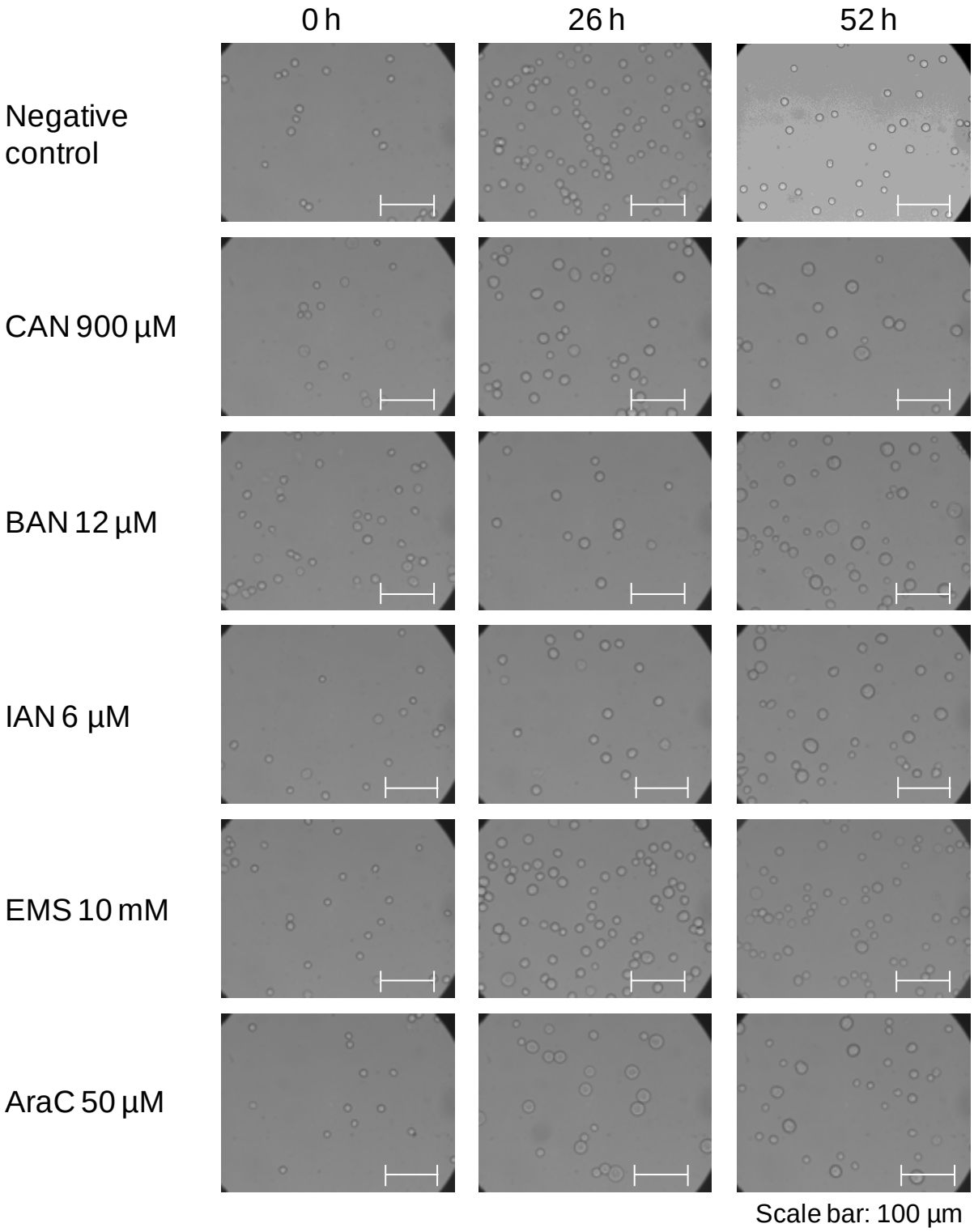

Figure 4.11 Microscopic images of harvested cells with 40× bright field illumination objective. CHO cells were harvested and observed at 0, 26, and 52 hour post-incubation. Treatment conditions are negative control, 900 µM CAN, 12 µM BAN, 6 µM IAN, 10 mM EMS or 50 µM AraC.

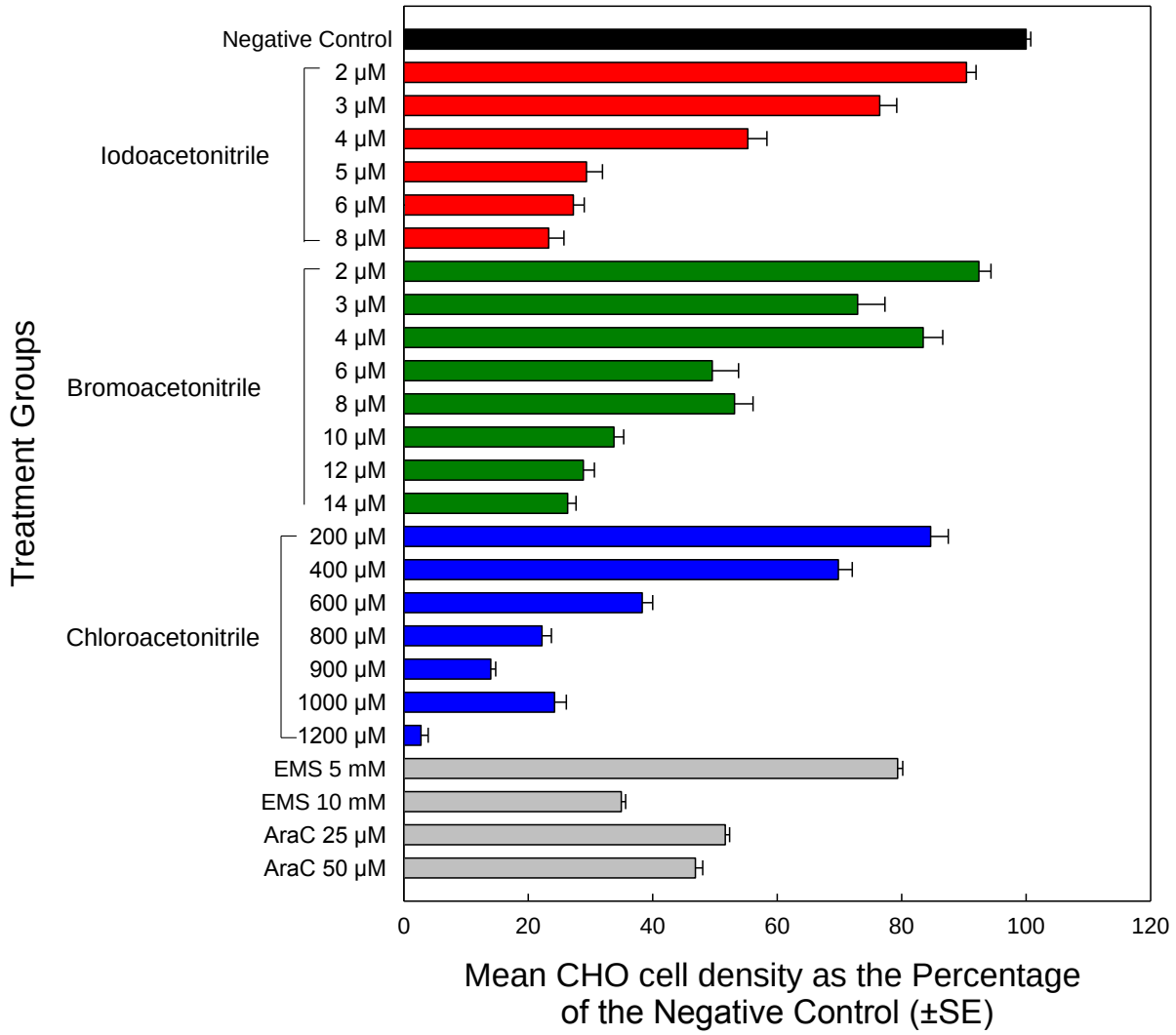

Figure 4.12 Cell density analysis of CHO cells exposed to HANs for 4 hours, washed, harvested and incubated (3,000 cell/well) for 72 hours. The positive controls were AraC and EMS.

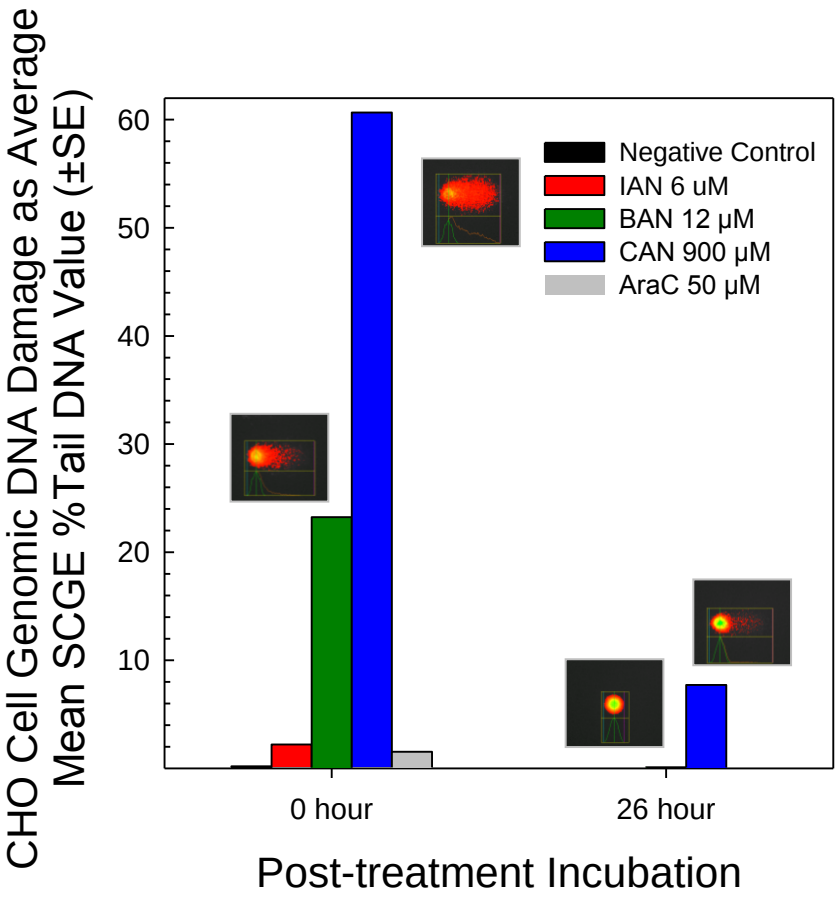

Figure 4.13 DNA strand breaks measured by single cell gel electrophoresis assay. Treated cells were harvested at 0 hour and 26 hour post-incubation.

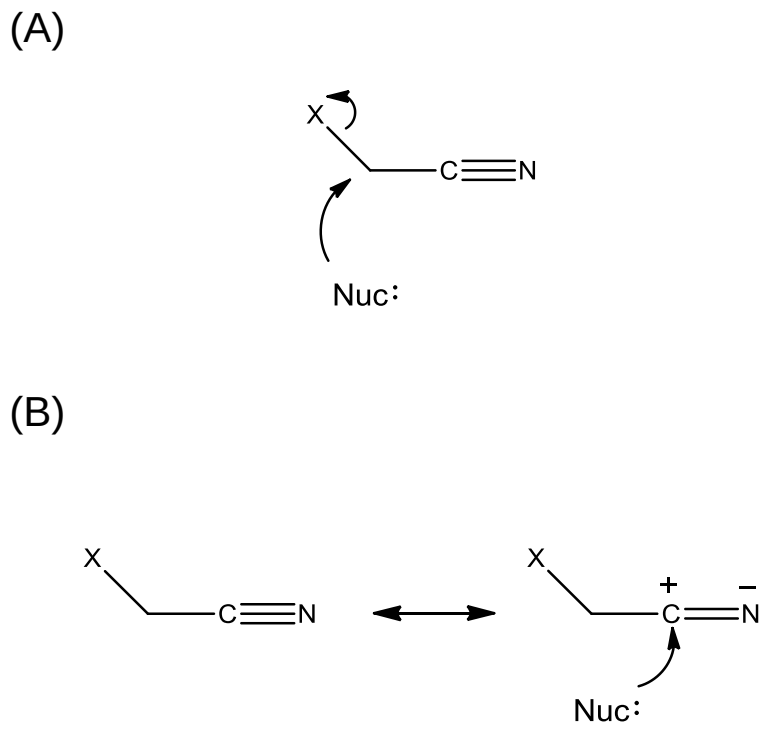

Figure 4.14 Two schemes for reactions of HANs with biological nucleophiles such as amino acids and DNA bases (Nuc:). (A) Displacement of halogen atom at α-carbon by S_{N2} substitution reaction. (B) Addition at the partially positively charged carbon atom of the cyano group.

CHAPTER 5

CELL CYCLE ALTERATION INDUCED BY MONOHALOACETIC ACIDS

ABSTRACT

Haloacetic acids (HAAs) and haloacetonitriles (HANs) are commonly found disinfection byproducts (DBPs) in drinking water. MonoHAAs and monoHANs have a similar chemical structure; both groups have a single halogen substituent at their α-carbon. They both induced genomic DNA damage with the rank order of iodinated > brominated > chlorinated analogues but repair efficiency of the induced lesions did not follow the same halogen order between these two groups. MonoHANs were shown to induce abnormally high DNA content in treated Chinese hamster ovary (CHO) cells and monoHAAs were expected to induce cell cycle delay as well. CHO cells were treated with chloroacetic acid, bromoacetic acid and iodoacetic acid using a wide range of concentrations followed by incubation in fresh medium to allow for cell accumulation. Cell cycle profile of HAA-treated cells was not notably different from negative control cells. Accumulation of abnormally high DNA content was not observed in the HAA-treated cells. Differences between treatment with monoHAAs and monoHANs in both DNA repair kinetics and cell cycle alteration suggested a fundamental difference in the mechanism of toxic action of these two classes of DBPs.

5.1 Introduction

Chemical disinfectants has been used as an effective defense against pathogens, yet because of their oxidizing potency they also react with ubiquitous natural organic matter, anthropogenic contaminants and bromide/iodide to form environmentally hazardous chemicals called disinfection byproducts (DBPs) [1]. Chlorine, chloramines, ozone and chlorine dioxide are common water disinfectants used today and they produce DBPs with different distributions [2]. In chlorinated water, haloacetic acids (HAAs) are the second most common class of DBPs after trihalomethanes [3]. The sum of five HAAs, chloroacetic acid

(CAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromoacetic acid (BAA) and dibromoacetic acid (DBAA), is regulated at a maximum contaminant level of 60 μg/L [4, 5]. HAAs are mutagenic in bacteria [6] and mammalian cells [7], induce genomic DNA damage in mammalian cells *in vitro* [8-11] and are developmentally toxic [12-14]. Carcinogenicity has been proven for DCAA, TCAA, bromochloroacetic acid (BCAA) and DBAA, but not for CAA [15-18]. Recently tumorgenicity of iodoacetic acid (IAA) was established [19].

Monohaloacetic acids have a single halogen substituent at the α-carbon. The association of toxic potency and halogen substitution was first realized by Richard and Hunter, with the rank order of IAA> BAA > CAA > fluoroacetic acid for their developmental toxicity [14]. Subsequently, the level of cytotoxic and genotoxic potency was found to follow the rank order of IAA > BAA > CAA in bacteria [6], Chinese hamster ovary (CHO) cells [7, 8] and human cells [11]. The potency of peroxisome proliferator-activated receptor α activation also followed the order of IAA > BAA > CAA in a primary hepatocytes culture [20]. MonoHAAs inhibits the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity in a concentration-dependent manner with the rank order of IAA > BAA >> CAA [21, 22]. The rate of inhibition of GAPDH and the genotoxic potency of HAAs were highly correlated, which suggested that GAPDH inhibition, the reduction of pyruvate and ATP levels, and the generation of reactive oxygen species induced as a mode of cytotoxic and genotoxic action of monoHAAs [21-23].

Attene-Ramos et al. [11] conducted a human cell toxicogenomic analysis of monoHAAs focusing on DNA damage signaling pathways. Transcriptome profiles impacted by the monoHAAs included genes related to double strand DNA break repair, cell cycle arrest, and apoptosis regulation. The number of modulated genes involving cell cycle regulation and apoptosis control was the highest among those with altered gene expression. In response to DNA damage, cells can activate checkpoints at critical stages in the cell cycle that prevent cells from progressing through the cell cycle [24, 25]. In this way, there is a longer period available for DNA repair prior to replication, leading to a suppression of mutation frequency [26]. Induction of abnormally high DNA content was observed in monohaloacetonitriles (monoHANs)-treated cells (Chapter 4). MonoHAAs and monoHANs have a similar chemical structure in

that both chemical groups have a single halogen substituent at the α-carbon. However, although they induce genomic DNA strand breaks with the same rank order of iodinated > brominated > chlorinated analogues [8, 27], they were shown to produce different DNA repair kinetics and associated effect of the halogen leaving group (Chapter 3). Cell cycle alteration was observed with HAN-treated cells (Chapter 4) and thus it would be interesting to study if differences in altered cell cycle progression in HAA-treated cells would reflect the differences in DNA repair kinetics. In this study, CHO cells were treated with monoHAAs and cell cycle profiles were determined using flow cytometry. Flow cytometry can quantify dye incorporation to DNA, which reflect the progression in mammalian cell cycle [28]. The objective of this study was to determine if monoHAAs alter the cell cycle of CHO cells and if that cell cycle alteration resembles HAN-induced cell cycle alteration.

5.2 Materials and Methods

5.2.1 Reagents

General reagents were purchased from Fisher Scientific Co. (Itasca, IL) and Sigma-Aldrich Co. (St. Louis, MO). Bromoacetic acid (≥ 99%, CAS# 79-08-3) and chloroacetic acid (≥ 99%, CAS# 79-11-8) were purchased from Fluka Chemical Co. (Buchs, Switzerland). Iodoacetic acid (≥ 99%, CAS# 64-69-7) was purchased from Aldrich Chemical Co. (Milwaukee, WI). HANs and HAAs were diluted in dimethyl sulfoxide (DMSO) at 1 mole/L and stored at −22°C.

5.2.2 Chinese hamster ovary cells and culture media

Transgenic Chinese hamster ovary (CHO) cells line AS52 clone 11-4-8 [29, 30] were maintained in modified Ham's F12 medium (Mediatech, Inc., Manassas, VA) supplemented with 5% fetal bovine serum (FBS), 1% L-glutamine and 1% antibiotic-antimycotic solution (10 units/mL penicillin G sodium, 10 μg/mL streptomycin sulfate, 25 μg/mL amphotericin B, 0.85% saline; Invitrogen, Carlsbad, CA). The cells exhibit normal morphology, express cell contact inhibition and grow as a monolayer without

expressing neoplastic foci. The cells were grown in 100-mm glass culture plate or 75-cm^2 tissue culture flask at 37°C in a humidified atmosphere of 5% CO_2.

5.2.3 Cell treatment and flow cytometric analysis of cell cycle

Unsynchronous CHO cells were plated in sterile flat-bottom 6-well tissue culture plates at 2×10^5/well/(2 mL F12+5% FBS). After 16 – 20 hours of incubation, cells were rinsed twice with 1 mL of Ca^{2+}, Mg^{2+} free-Hank's balanced salt solution (HBSS), and treated with each chemical in 1 mL of serum-free F12 medium for 4 hours at 37°C, 5% CO_2. Multiple wells were used per treatment group as needed. A sheet of sterile AlumnaSeal™ (RPI Corporation, Mt. Prospect, IL) was pressed over the wells before covering the plate with a lid to prevent crossover contamination between wells due to volatilization. After the 4-hour treatment, the solution was aspirated, and the cells were washed twice with 1 mL HBSS. The cells were harvested immediately (0 hour sample) with 0.5× trypsin (0.05% trypsin, in HBSS with 0.2 g/L EDTA, without calcium, magnesium, Hyclone Laboratories, South Logan, UT), or 2 mL of drug-free F12+5% FBS were added and incubated for designated times. The post-treatment incubation times allowed cells to accumulate at intervals where there was cell cycle blockage. Harvested cells were counted with Beckman Coulter Z1 Particle Counter (Beckman Coulter, Inc., Brea CA) and also acute cytotoxicity was determined using the trypan blue dye exclusion assay [31]. In order to determine their DNA content, harvested cells were fixed in 70% ice cold ethanol. Samples were stored for up to one week at −22°C. The fixed cells were rinsed with cold phosphate buffered saline (PBS) twice and then incubated for 30 minutes at 37°C with 50 µg/mL of the DNA fluorochrome propidium iodide, a marker of DNA content, in a solution containing 200 µg/mL of RNase A. Cell fluorescence was measured in the Biosciences LSR II flow cytometry analyzer (San Jose, CA). Propidium iodide was excited by a 488 nm laser line (laser diode) and its emission was collected through a 695/40 nm band-pass filter. The data were analyzed using multicycle DNA analysis using FCS Express version 4 (De Novo Software, Los Angeles, CA) and aggregated cells were gated out.

5.2.4 Plating efficiency

CHO cells were treated in 6-well tissue culture plates in the same way as for cell cycle analysis. After a 4-hour treatment, the solution was aspirated, and the cells were washed twice with 1 mL HBSS and harvested with 0.025% trypsin + 0.01% EDTA in HBSS. The cell number was counted with Coulter counter and adjusted to 3×10^4 cells/mL. In the flat-bottom, tissue culture 96-well microplate, one column of eight wells was reserved for the blank control consisting of 200 µL of F12+5% FBS. Titered cells of 100 µL were aliquoted into one column plus 100 µL F12+5% FBS. To prevent volatilization of culture medium, a sheet of sterile AlumnaSeal™ was pressed over the wells before covering the microplate with a lid. The microplate was placed on a rocking platform for 10 minutes to uniformly distribute the cells, and then it was placed in a tissue culture incubator for 72 hours. After incubation, each well was gently aspirated, fixed in 100% methanol for 10 minutes, and stained for 10 min with a 1% crystal violet solution in 50% methanol. The plate was gently washed, and 50 µL of a DMSO:methanol (3:1, v/v) solution was added to each well for 10 minutes. The plate was then analyzed in SpectraMax microplate reader (Molecular Devices, LLC, Sunnyvale, CA) at 595 nm. The blank-corrected absorbency value of the negative control was set at 100%. The absorbency for each treatment group well was converted into a percentage of the negative control. For each concentration, eight replicate wells were analyzed per experiment and each experiment was independently repeated at least three times.

5.2.5 Single cell gel electrophoresis

To examine the induction of genomic DNA damage in different physical conditions in 96-well microplates and 6-well plates, single cell gel electrophoresis (SCGE) assay was conducted in parallel. The SCGE assay is rapid and sensitive, and detects lesions including DNA single-strand and double-strand breaks, incomplete excision repair sites, and alkali-labile sites [32, 33]. Detailed methods for preparation of SCGE slides and electrophoresis have been published previously [8, 9, 34].

The day before treatment, CHO cells were plated in a sterile flat-bottom 96-well microplate at 4×10^4 CHO cells/(200 µL of F12+5% FBS)/well or in a sterile flat-bottom 6-well tissue culture plates at 2×10^5

cells/ (2 mL F12+5% FBS)/well. The next day, the cells were washed with HBSS and treated with the HAAs in serum-free F12 medium in a total volume of 25 μL in 96-well microplate and 1 mL in 6-well plate in parallel for 4 hours at 37°C, 5% CO_2. The wells were covered with AlumnaSeal™ to prevent chemical volatilization. After treatment, the solution was aspirated and the cells were washed twice with HBSS. The cells in 96-well microplate were detached with 50 μL of a 0.025% trypsin+0.01% EDTA solution in HBSS, followed by addition of 70 μL F12+5% FBS. A 10 μL aliquot was analyzed for acute cytotoxicity with the trypan blue dye exclusion assay [31] to ensure that cell viability exceeds 70% [35]. The remaining cell suspension was mixed with the same volume of 1% of low melting point agarose (LMA), and layered onto clear microscope slides pre-coated with 1% normal melting point agarose. The cells in 6-well plate were detached with 400 μL of a 0.025% trypsin+0.01% EDTA solution in HBSS, followed by addition of 1 mL F12+5% FBS. A 120 μL of cell suspension was mixed with 120 μL of 1 % LMA and applied onto pre-coated microscope slides. After the first layer of LMA solidified, another layer of 0.5% LMA was applied. Two SCGE microgels were prepared from the suspension harvested from each well. The microgels were immersed in lysing solution (2.5 M NaCl, 1% sodium lauryl sarcosinate, 100 mM Na_2EDTA, 10 mM Tris, pH 10 with 1% Triton X-100 and 10% DMSO) overnight at 4°C.

The DNA of the nuclei in the SCGE microgels was denatured for 20 minutes in electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH 13.5) followed by electrophoresis at 25 V and 300 mA (0.72 V/cm) for 40 minutes at 4°C. After neutralization with 400 mM Tris buffer (pH 7.5) the microgels were dehydrated in cold methanol for 20 minutes, dried for 5 minutes at 50°C, and stored at room temperature. For analysis, the microgels were hydrated in water at 4°C for 20 minutes, and then the nuclei were stained with 65 μL of an ethidium bromide solution (20 μg/mL). Fifty randomly chosen nuclei per microgel were analyzed with a Zeiss fluorescence microscope (excitation filter of BP 546/10 nm, barrier filter of 590 nm) and a computerized image analysis system (Comet Assay IV, Perspective Instruments Ltd., Suffolk, UK). The digitalized data were automatically transferred from the CCD camera to a computer-based spreadsheet for statistical analysis. The percentage of migrated DNA out of nucleus (% tail DNA) was used as the primary measurement.

5.3 Results

5.3.1 Examination of HAA concentration for cell cycle analysis

The initial loading of cells and post-incubation time for cell cycle observation were determined previously (Chapter 4). The duration of 26 hour post-incubation allows cells to accumulate where cell cycle is delayed or blocked and the initial cell loading of 2×10^5 cells/well ensures the exponential cell growth up to 26 hours. The HAA concentrations that were used for DNA repair kinetics were 6 mM CAA, 60 µM BAA and 25 µM IAA [36]. These concentrations were used as a starting point. However, no notable cell cycle alteration was observed with these concentrations. Thus to determine the optimum HAA concentration for cell cycle experiments, CHO cells were treated with a wide range of HAA concentrations and cell cycle of HAA-treated cells after 26 hour post-incubation was analyzed. Figure 5.1 displays the cell cycle profile obtained with the LSR II flow cytometer as well as cell cycle distribution calculated using the FCS Express software. Cell cycle distribution is displayed by DNA content as 2N, 2N-4N and 4N, which correspond to cells in G_1, S and G_2/M phase, respectively. With CAA at 2 – 8 mM, BAA at 30 – 150 µM, and IAA at 10 – 60 µM, no notable cell cycle alteration was observed compared to concurrent negative controls (Figure 5.1). The concentration of BAA was raised up to 500 µM. The 4N cell population increased with BAA concentration: 4.70% (0 µM), 3.14 % (100 µM), 10.5% (200 µM), 9.79% (300 µM), and 17.4% (400 µM), 17.9% (500 µM) (Figure 5.2).

5.3.2 Examination of the duration of post-incubation for cell cycle analysis

Although the duration of post-incubation was determined as 26 hours previously and that observation window allowed for detection of cell cycle alteration by HANs (Chapter 4), the duration of post-incubation was re-examined with IAA. CHO cells were treated with IAA at 50 µM for 4 hours in a 6-well plate. Treated cells were washed twice with HBSS and fresh F12+5% FBS was added. After 4 and 8 hours, the cells were collected and the cell cycle profile was analyzed (Figure 5.3). The cell cycle population was 47.8% (2N), 12.3% (2N-4N), 39.9% (4N) for negative control cells and 44.3% (2N), 15.3% (2N-4N), and 40.3% (4N) for IAA-treated cells at 4 hour post-incubation and they were not

notably different as observed in HAN-treated cells. Similarly, difference was not observed in cell cycle population at 8 hour post-incubation; the cell cycle population with 2N, 2N-4N and 4N content were 45.4%, 47.5%, 7.1%, respectively, for negative control cells, and 44.7%, 45.9%, and 9.4%, respectively, for IAA-treated cells.

5.3.3 Plating efficiency

CHO cells were treated in the 6-well tissue culture plate for 4 hours, washed and harvested. Equal number of treated cells was plated onto a new 96-well microplate and the resulting cell densities after 72 hours were compared to their concurrent negative controls. Within the concentrations tested, CAA and IAA did not suppress the cell growth except for the highest concentration used. The mean ± standard error (SE) cell densities compared to their concurrent negative controls was 81.2 ± 1.8% for CAA at 8 mM and 84.6 ± 4.8% for IAA at 30 µM. On the other hand, BAA-treated cells demonstrated repressed cell growth over the concentration range tested; the resulting cell densities compared to the negative control was 94.5 ± 4.7% (30 µM), 72.9 ± 6.0% (60 µM), 52.6 ± 3.1% (90 µM), 56.3 ± 3.6% (120 µM) and 50.0 ± 4.2% (150 µM) (Figure 5.4).

5.3.4 DNA strand breaks in different test systems

SCGE assay was conducted with CHO cells treated in a 96-well microplate with high cell density (the same condition as the genomic DNA damage induction and DNA repair kinetics) and in a 6-well plate with low cell density (the same condition as cell cycle analysis). The genomic DNA induction was generally lower when cells were treated in 6-well plate with low cell density. At the highest concentrations tested, CAA at 7 mM induced % tail DNA of 8.38 and 4.85 (Figure 5.5), BAA at 100 µM induced % tail DNA of 22.4 and 2.45 (Figure 5.6), and IAA at 30 µM induced % tail DNA of 26.8 and 21.7 (Figure 5.7), at high cell density and low density, respectively.

5.4 Discussion

Eukaryotic cells have multiple checkpoints to ensure the integrity of the genome and cell division [37-39]. DNA integrity checkpoints monitor DNA status and prevent inappropriate transitions into S phase and mitosis when aberrations are detected [24, 25]. Recent human cell toxicogenomic analysis focusing DNA damage signaling pathway demonstrated that gene expression involving cell cycle regulation was most affected [11]. Also, delayed cell cycle progression was reflected in the cell growth suppression in HAA-treated human cells. These results provided motivation to proceed to the cell cycle analysis of HAA-affected cells.

Chapter 4 demonstrated that the monoHANs induced abnormally high DNA content in treated cells. MonoHAAs and monoHANs have similar chemical structure in that both chemical groups have a single halogen substituent at the α-carbon. They both induced genomic DNA strand breaks with the rank order of iodinated > brominated > chlorinated analogues [8, 27] but induced DNA lesions were repaired with different efficiency (Chapter 2, 3). Thus we were interest if different cell cycle alteration can be observed in HAA-treated cells as reflected by the different DNA repair kinetics. We expected stronger cell cycle arrest in HAA-treated cells due to the associated DNA lesions that should cause longer processing time. Contrary to the anticipation, HAAs did not produce as distinct cell cycle alteration when compared to those by HANs; abnormally high DNA content or changes in cell ploidy was not observed in HAA-treated cells in any of the conditions tested, which suggested a fundamentally different molecular mechanism of toxic action between HAAs and HANs.

It was suggested that HAN toxicity was dependent on mitosis and proliferation (Chapter 4). The treatment of high-density CHO cells with chloroacetonitrile (CAN) at 1200 μM, bromoacetonitrile (BAN) at 60 μM and iodoacetonitrile (IAN) at 60 μM followed by incubation in serum-free medium did not kill the cells after 24 hours (Chapter 3), but the treatment concentrations needed to be lowered to 900 μM CAN, 12 μM BAN and 6 μM IAN to avoid excessive cell killing when the cells were treated at low density followed by incubation in medium with serum for 26 hours (Chapter 4). Cell cycle progression was suppressed in the former case. This suggested the proliferation dependence of HAN toxicity.

It appeared that the effect of cell proliferation state was completely opposite for HAA toxicity; the toxic potency of HAAs was weaker when cells were treated at low density. HAA treatment of CHO cells at low cell density did not repress the cell growth as much as when cells were treated at high cell density. The plating efficiency of HAA-treated cells at low density in a 6-well plate was 97.1 ± 1.4% for CAA at 6 mM, 72.9 ± 6.0% for BAA at 60 µM, and 96.9 ± 4.0% for IAA at 25 µM. When plated cells were confluent at the treatment, the plating efficiency was 56.0 ± 0.9% for CAA at 6 mM, 74.4 ± 2.9% for BAA at 60 µM, and 60.4 ± 4.1% for IAA at 25 µM (Chapter 3). BAA-treated cells had the similar plating efficiency in these two different treatment conditions, but when cells were treated at low density, IAA and CAA did not suppress the cell growth.

In order to further examine if the toxic potency of HAAs was weaker when cells were treated at low density, genomic DNA damage induction was measured using SCGE assay. The day before treatment, CHO cells were plated in a sterile flat-bottom 96-well microplate at 4×10^4 CHO cells/(200 µL of F12+5% FBS)/well or in a sterile flat-bottom 6-well tissue culture plates at 2×10^5 cells/(2 mL F12+5% FBS)/well. Initial cell density in 96-well microplate was 4×10^4 cells/0.32 cm^2/96-well (= 1.25×10^5 cells/cm^2) whereas initial cell density in 6-well plate was 2×10^5 cells/9.5 cm^2/6-well (= 2.11×10^4 cells/cm^2), respectively. After 16 – 20 hour incubation, the cells were treated with HAAs at various concentrations. The variables were minimized by setting up the 96-well microplate and 6-well plate on the same day using the same cell suspension, and by treating the cells at the same time with the same chemical dilution. The genomic DNA induction was generally lower when cells were treated in 6-well plate with low cell density (Figures 5.5, 5.6, and 5.7).

There is an evidence that HAAs do not directly interact with genomic DNA but generate reactive oxygen species (ROS) that leads to induction of toxicity and DNA damage. The co-treatment of IAA with antioxidants reduced the IAA-mediated mutagenicity in *Salmonella typhimurium* and genomic DNA damage induction in CHO cells [40]. Dibromoacetic acid and bromochloroacetic acid induced oxidative stress in male B6C3F1 mice [41]. MonoHAAs was shown to inhibit glyceraldehydes-3-phosphate dehydrogenase [21, 22], which may lead to ROS production. It is possible that the low cell density state

as in the 6-well plate dissipated the ROS into the system due to the higher availability of medium per cells. Also, excess availability of pyruvate in F12 medium may have attenuated monoHAA-induced genomic DNA. Future experiments are required to understand this result.

In conclusion, cell cycle alteration was not strongly observed in monoHAA-treated cells. Since monoHAA-induced DNA lesions required longer processing time than monoHAN-induced lesions as demonstrated in Chapter 2 and 3, strong cell cycle delay was expected in HAA-treated cells. The difference of monoHAAs and monoHANs in both DNA repair kinetics and cell cycle alteration suggested fundamentally different mechanisms of toxic of action of these two classes of DBPs.

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Tables and Figures

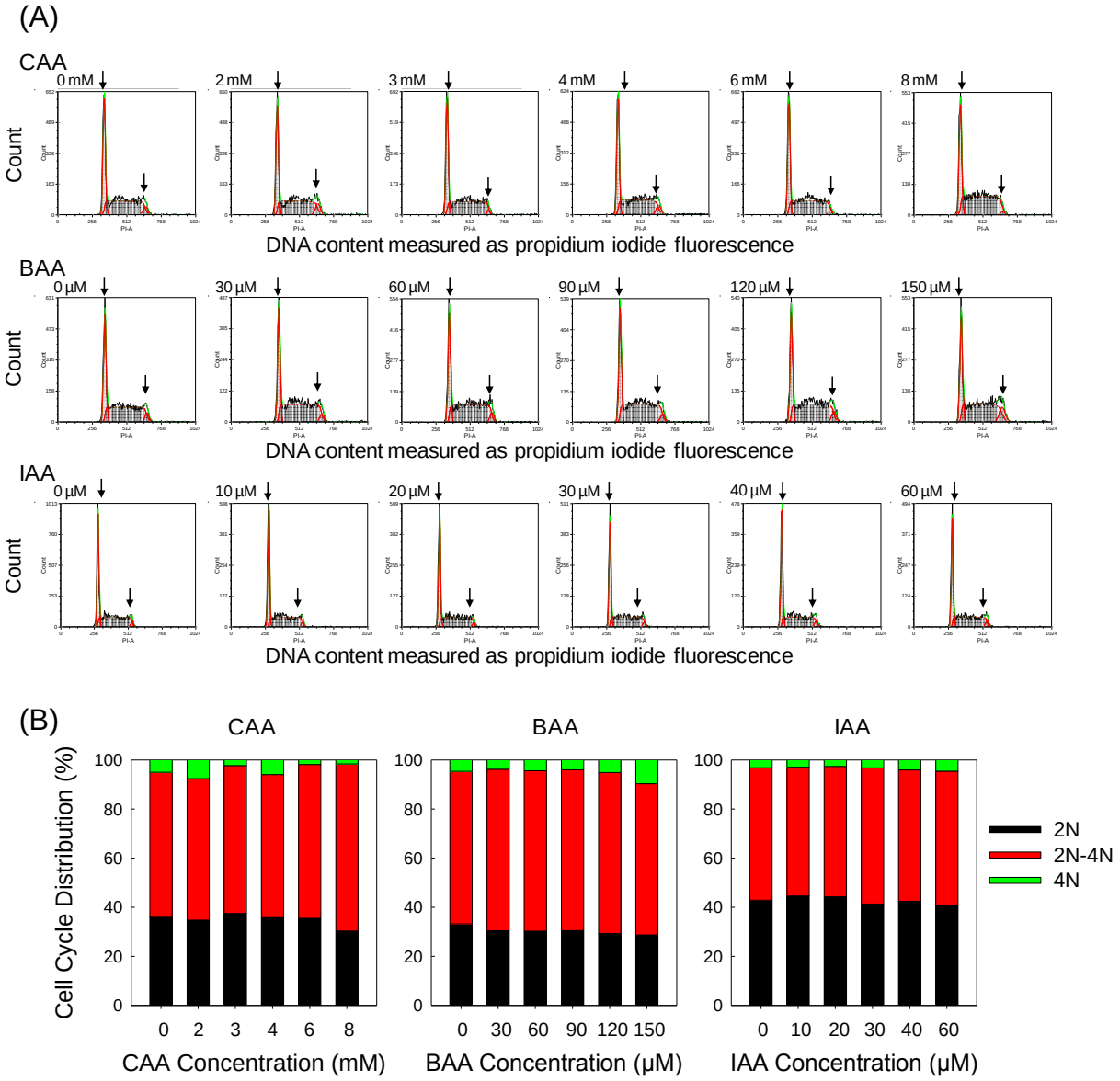
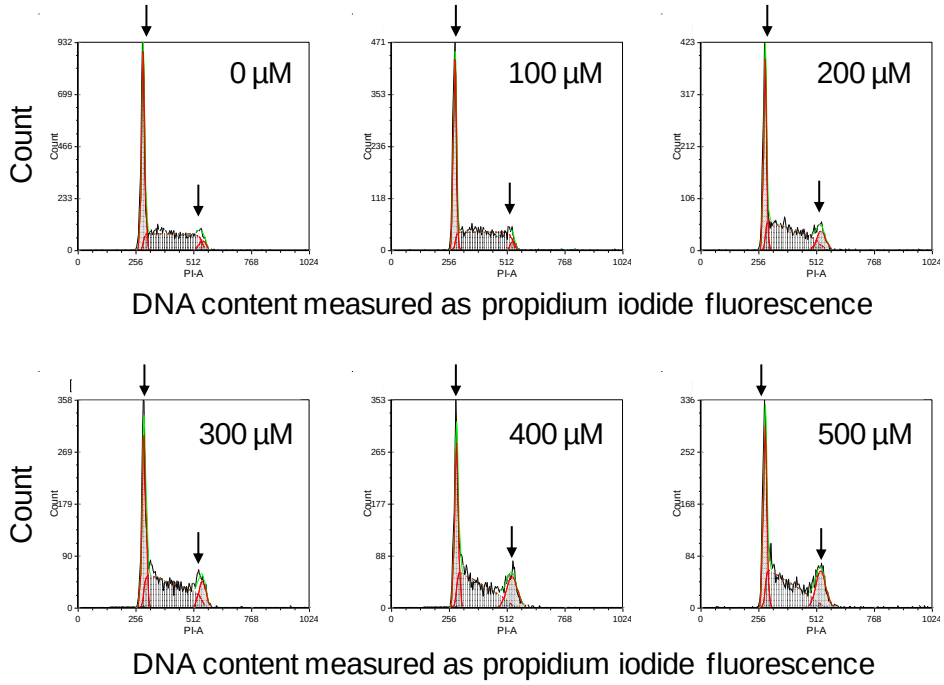


Figure 5.1 HAA concentration optimization. (A) Cell cycle profiles obtained with LSR II flow cytometer. CAA (top), BAA (center), IAA (bottom). Arrows indicate the 2N and 4N DNA containing cells from the left. (B) Cell cycle distribution calculated with the FCS Express software.

(A)



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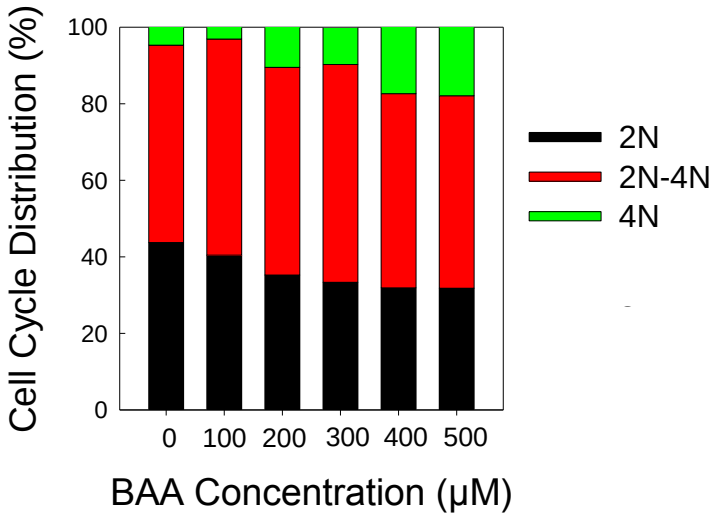

Figure 5.2 BAA concentration was raised up to 500 µM. (A) Cell cycle profile obtained with the LSR II flow cytometer. Arrows indicate the 2N and 4N DNA containing cells from the left. (B) The cell cycle distribution calculated with the FCS Express software.

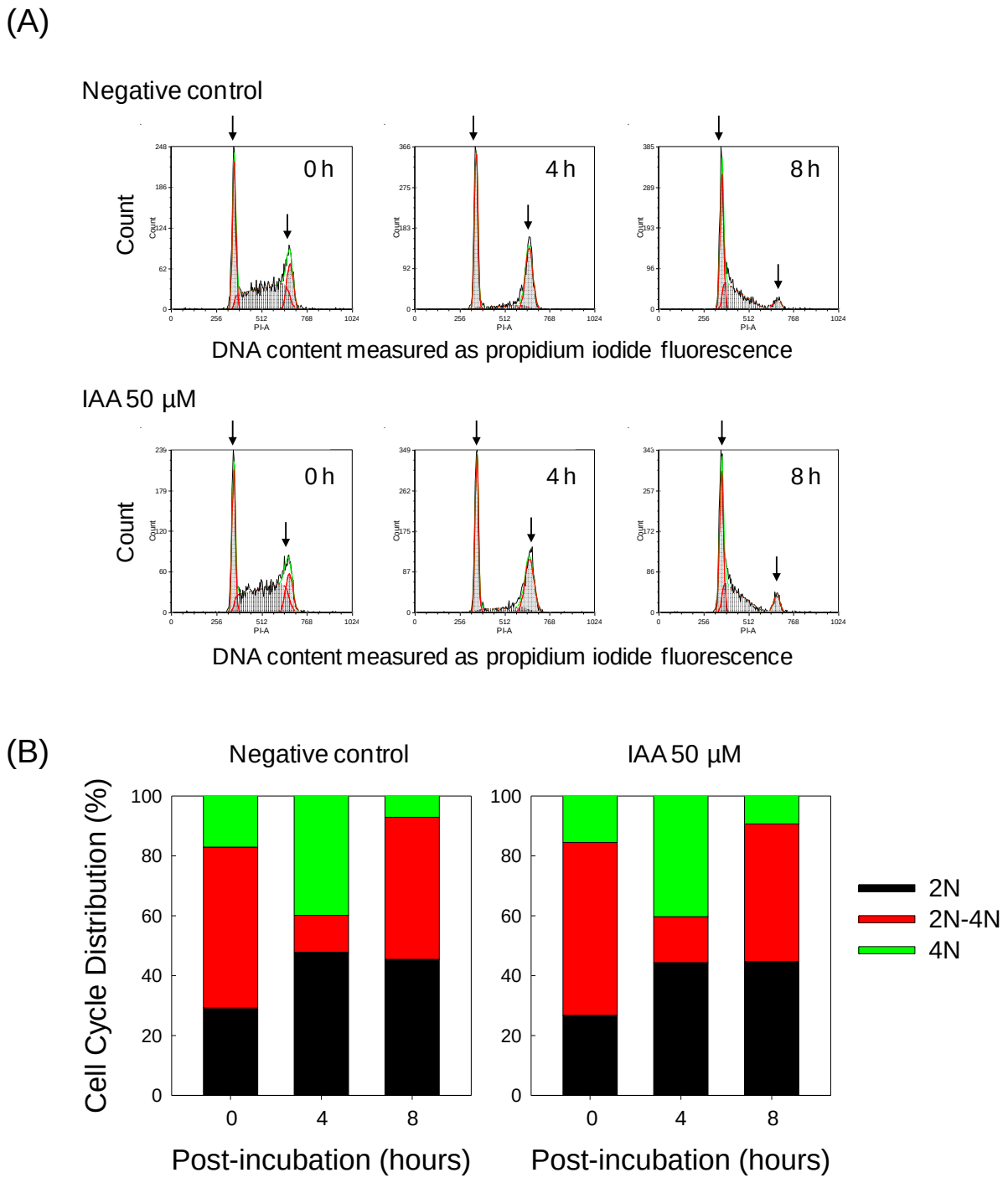

Figure 5.3 The duration of post-incubation of 4 and 8 hours was examined after treatment with IAA at 50 µM. (A) Cell cycle profile obtained with LSR II flow cytometer. Arrows indicate the 2N and 4N DNA containing cells from the left. (B) The cell cycle distribution calculated with the FCS Express software.

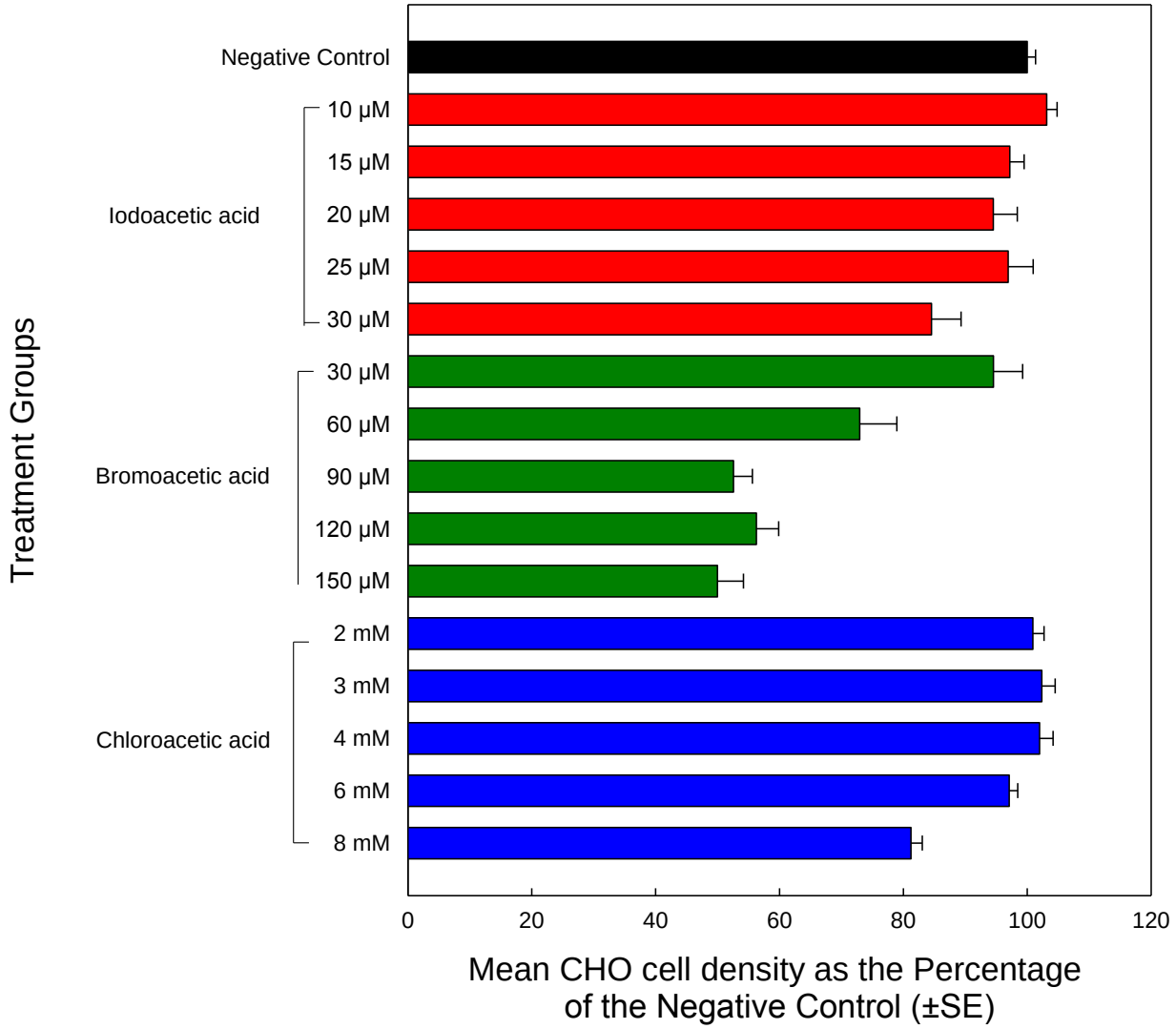

Figure 5.4 Cell density analysis of CHO cells exposed to HAAs for 4 hours, washed and harvested, and incubated in a 96-well microplate (3,000 cells/well) for 72 hours.

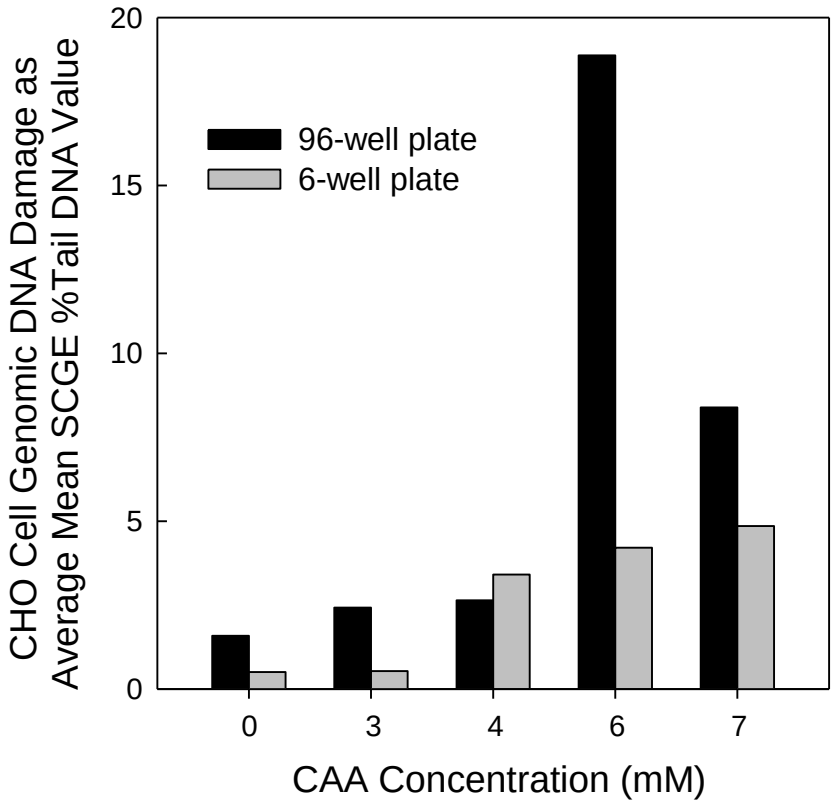

Figure 5.5 CAA-mediated genomic DNA damage measured with single cell gel electrophoresis assay. CHO cells were treated in high density (in 96-well microplate) and low density (in 6-well plate).

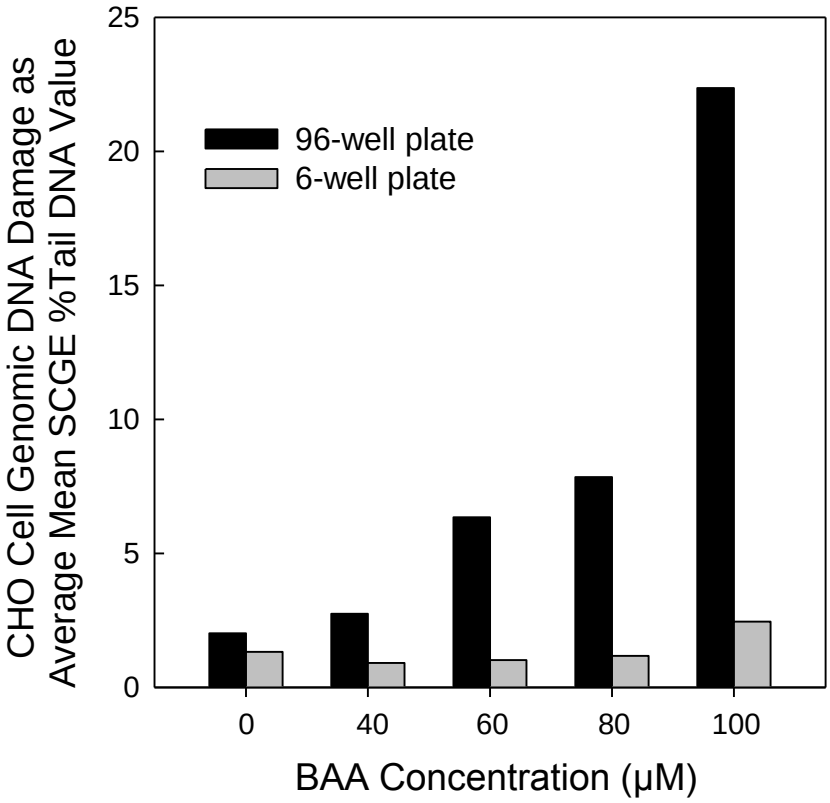

Figure 5.6 BAA-mediated genomic DNA damage measured with single cell gel electrophoresis assay. CHO cells were treated in high density (in 96-well microplate) and low density (in 6-well plate).

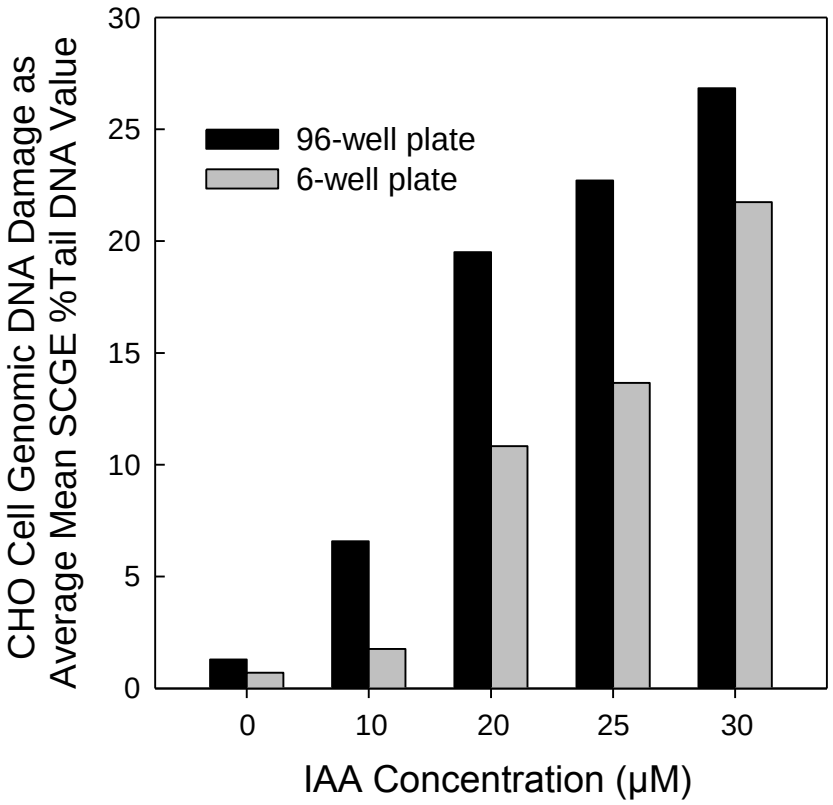

Figure 5.7 IAA-mediated genomic DNA damage measured with single cell gel electrophoresis assay. CHO cells were treated in high density (in 96-well microplate) and low density (in 6-well plate).

CHAPTER 6

CONCLUSIONS

This research aimed to compare the toxic properties of haloacetic acids (HAAs) and haloacetonitriles (HANs), as representative carbonaceous and nitrogenous DBPs using Chinese hamster ovary cell as a model mammalian cell system. These classes of DBPs are commonly found in drinking water and both classes are mutagenic, genotoxic, teratogenic and developmentally toxic in a wide variety of biological systems. MonoHAAs and monoHANs have a similar chemical structure, a single halogen substituent at their α-carbon, where substitution reactions can occur with a halogen atom as a leaving group. Both the HAAs and HANs induce genomic DNA damage with the rank order of iodinated > brominated > chlorinated analogues. DNA damage in eukaryotic cells is strongly associated with DNA repair and cell cycle progression. In order to provide insights on the mechanism of toxic action of these two classes of DBPs, the repair rate of induced DNA lesions and the occurrence of cell cycle alterations were examined.

The microplate-based single cell gel electrophoresis assay was modified to include liquid holding recovery to measure the reduction of genomic DNA damage level over time. The DNA lesions induced by monoHANs were repaired more efficiently than HAA-induced lesions. Within HAAs, BAA-induced lesions were repaired least efficiently compared to those induced by IAA and CAA. HAN-induced lesions did not express constant differences in the repair efficiency among the three homologs. Also the impact of halogen atom substitution was not consistent between two chemical classes.

Cytometric analysis demonstrated remarkable differences in the effects on cell cycle progression induced by the monoHAAs and monoHANs on post-treatment CHO cells. HAN-induced cell cycle modulation was concentration-dependent and also followed the genotoxicity rank order pattern of I > Br > Cl. HAN-treated cells accumulated cells with abnormally high levels of DNA content and had repressed cell growth. However, DNA strand breaks were not present when cell cycle alteration was observed during post-incubation. HAN-mediated cell cycle was mitosis- and proliferation-dependent, which suggested that HAN treatment induced mitotic failure in CHO cells and DNA content accumulation in the

HAN-treated cells. However, HAA-treated cells did not demonstrate any significant effect on post-treatment cell cycle progression. The difference of monoHAAs and monoHANs in both DNA repair kinetics and cell cycle alterations indicate that fundamental differences in the mechanism of toxic action exist for these classes of DBPs.

This study demonstrated the efficacy provided by the use of multiple toxicity endpoints to address possible adverse health outcomes associated with exposure to disinfection byproducts. The experimental designs developed for the DNA repair kinetics and cell cycle alteration may lead to novel toxicity endpoints that could be incorporated in the battery of DBP toxicity evaluation. The severity of cell cycle modulation and the resulting aneuploidy of HAN-treated cells indicate that HANs may play a major role in cancer induction and adverse pregnancy outcomes associated with long-term exposure to disinfected drinking water. With the increasing stress on source waters due to water scarcity, many utilities seek alternative disinfection processes or alterations to chlorination practices. Regulated DBPs currently are employed as indicators for DBP control but many scientists believe that these regulated DBPs do not fully account for the adverse health outcomes identified by epidemiology studies. Identification of major DBP classes associated with adverse health outcomes from disinfected drinking water will aid in developing science-based DBP control strategy for drinking water treatment to enhance the public health and the environment protection.

APPENDIX A

APPENDICES FOR CHAPTER 2

Summary of contents:

- Fifty nine pages.
- Table A.1: Raw data of cell viability throughout a 60-hour time period with cells maintained under liquid holding conditions.
- Table A.2: Regression analysis of the CHO cell viability data with the 60-hour liquid holding.
- Table A.3: Raw data of the comparative genomic DNA repair kinetics in CHO cells for chloroacetic acid (CAA), iodoacetic acid (IAA), and bromoacetic acid (BAA).
- Table A.4: Regression analysis of the CHO cell CAA DNA repair kinetics data.
- Table A.5: Regression analysis of the CHO cell BAA DNA repair kinetics data.
- Table A.6: Regression analysis of the CHO cell IAA DNA repair kinetics data.
- Table A.7: Two-way Analysis of Variance for DNA repair of CHO cells treated with chloroacetic acid (CAA), bromoacetic acid (BAA) and iodoacetic acid (IAA).
- Table A.8: Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with chloroacetic acid without and with 1, 2, 3, 4, 5 and 24 hour liquid-holding recovery.
- Table A.9: Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with bromoacetic acid without and with 1, 2, 3, 4, 5 and 24 hour liquid-holding recovery.
- Table A.10: Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with iodoacetic acid without and with 1, 2, 3, 4, 5 and 24 hour liquid-holding recovery.

- Table A.11: Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from the CHO cell negative controls at 0, 5, and 24 hour liquid-holding recovery.

Table A.1 Raw data of cell viability throughout a 60-hour time period with cells maintained under liquid holding conditions. Cell numbers were counted in triplicate.

Liquid holding time (hours)	Live	Dead	Live	Dead	Live	Dead	% Cell viability
0	131	7	114	14	129	15	91.22
8	100	4	131	5	109	2	96.87
26	113	10	141	7	116	2	95.12
32	148	16	116	4	129	14	92.04
38	131	14	112	14	124	7	91.29
48.5	116	14	126	10	108	11	90.91
51	113	16	127	12	119	23	87.56
53	106	11	106	8	137	20	89.95
60	112	49	138	40	116	44	73.35
60.5	143	36	105	38	123	39	76.65

Table A.2 Regression analysis of the CHO cell viability data with the 60-hour liquid holding.

Nonlinear Regression

[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
[Parameters]
a = max(y) "Auto {{previous: 93.5032}}
b = if(max(y)>0, xwtr(x,y,.5)/4, -xwtr(x,y,.5)/4) "Auto {{previous: -5.67684}}
x0 = x50(x,y,.5) "Auto {{previous: 68.2979}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=0.000100
stepsize=100
iterations=100
"iterations=0

R = 0.95742268 Rsqr = 0.91665818 Adj Rsqr = 0.89284623

Standard Error of Estimate = 2.4887

	Coefficient	Std. Error	t	P
a	93.5032	1.1937	78.3304	<0.0001
b	-5.6768	1.5892	-3.5721	0.0091
x0	68.2979	2.3645	28.8852	<0.0001

Analysis of Variance:

	DF	SS	MS	F	P
Regression	2	476.8749	238.4375	38.4957	0.0002
Residual	7	43.3571	6.1939		
Total	9	520.2320	57.8036		

PRESS = 104.3011

Durbin-Watson Statistic = 2.6076

Normality Test: K-S Statistic = 0.2246 Significance Level = 0.6353

Constant Variance Test: Passed (P = 0.8379)

Power of performed test with alpha = 0.0500: 0.9990

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	93.5026	-2.2831	-0.9174	-1.0452	-1.0534
2	93.5009	3.3652	1.3522	1.5396	1.7527
3	93.4489	1.6668	0.6697	0.7534	0.7276

Table A.2 (cont.)

4	93.3471	-1.3097	-0.5262	-0.5832	-0.5536
5	93.0556	-1.7621	-0.7080	-0.7684	-0.7434
6	90.7289	0.1802	0.0724	0.0833	0.0772
7	89.2635	-1.7025	-0.6841	-0.8420	-0.8223
8	87.5862	2.3623	0.9492	1.2330	1.2902
9	75.9054	-2.5587	-1.0281	-1.3819	-1.5003
10	74.6125	2.0404	0.8199	1.2311	1.2877

Influence Diagnostics:

Row	**Cook'sDist**	**Leverage**	**DFFITS**
1	0.1086	0.2297	-0.5752
2	0.2343	0.2287	0.9544
3	0.0502	0.2097	0.3748
4	0.0259	0.1859	-0.2645
5	0.0350	0.1510	-0.3135
6	0.0007	0.2447	0.0439
7	0.1217	0.3400	-0.5902
8	0.3484	0.4074	1.0698
9	0.5135	0.4465	-1.3475
10	0.6340	0.5565	1.4425

95% Confidence:

Row	**Predicted**	**Regr. 5%**	**Regr. 95%**	**Pop. 5%**	**Pop. 95%**
1	93.5026	90.6823	96.3229	86.9768	100.0285
2	93.5009	90.6867	96.3151	86.9777	100.0241
3	93.4489	90.7541	96.1437	86.9763	99.9215
4	93.3471	90.8099	95.8844	86.9385	99.7557
5	93.0556	90.7690	95.3422	86.7420	99.3692
6	90.7289	87.8176	93.6402	84.1632	97.2946
7	89.2635	85.8321	92.6949	82.4512	96.0758
8	87.5862	83.8299	91.3425	80.6046	94.5678
9	75.9054	71.9731	79.8377	68.8276	82.9832
10	74.6125	70.2223	79.0027	67.2704	81.9546

Table A.3 Raw data of the comparative genomic DNA repair kinetics in CHO cells for chloroacetic acid (CAA), iodoacetic acid (IAA), and bromoacetic acid (BAA).

CAA 6 mM

Liquid holding time (hours)	%T compared to 0-hr	Standard error of the mean	% Cell viability
0	100.0000	1.7376	96.70
1	65.6467	3.6168	94.38
2	55.8418	4.7360	98.77
3	42.4971	6.7360	96.47
4	43.1029	2.3404	99.09
5	33.5174	7.1442	96.59
24	15.1674	4.2768	98.03

BAA 60 µM

Liquid holding time (hours)	%T compared to 0-hr	Standard error of the mean	% Cell viability
0	100.0000	0.9820	98.26
1	90.8643	4.8186	97.19
2	75.1934	11.4845	95.52
3	65.8421	6.7913	95.60
4	63.0035	3.9422	97.07
5	47.3868	3.3435	96.53
24	20.7838	2.3102	90.09

IAA 25 µM

Liquid holding time (hours)	%T compared to 0-hr	Standard error of the mean	% Cell viability
0	100.0000	2.2584	96.56
0.5	70.3823	8.5730	96.20
1	69.7274	6.8941	97.22
1.5	43.3533	2.2991	97.63
2	40.1440	4.7579	98.60
2.5	36.2164	5.4431	97.90
3	29.7061	4.2074	98.73
3.5	28.9711	2.2605	98.96
4	31.6431	2.3565	100.00
4.5	30.4182	2.0769	99.04
5	26.0237	2.5295	97.74
24	13.2242	2.1207	98.14

Table A.4 Regression analysis of the CHO cell CAA DNA repair kinetics data.

Nonlinear Regression

[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 4065.59}}
b = xwtr(x,y-min(y),.5)/4 "Auto {{previous: -2.79955}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: -11.0281}}
y0 = min(y) "Auto {{previous: 17.8924}}
[Equation]
f= y0+a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=0.000100
stepsize=100
iterations=100

R = 0.98542405 Rsqr = 0.97106056 Adj Rsqr = 0.94212113

Standard Error of Estimate = 6.4875

	Coefficient	Std. Error	t	P
a	4065.5904	198202.1554	0.0205	0.9849
b	-2.7995	2.0910	-1.3389	0.2730
x0	-11.0281	159.5618	-0.0691	0.9492
y0	17.8924	6.4971	2.7539	0.0705

Analysis of Variance:

	DF	SS	MS	F	P
Regression	3	4236.7713	1412.2571	33.5549	0.0083
Residual	3	126.2638	42.0879		
Total	6	4363.0350	727.1725		

PRESS = 88606.4738

Durbin-Watson Statistic = 2.3036

Normality Test: K-S Statistic = 0.1322 Significance Level = 0.9991

Constant Variance Test: Passed (P = 0.6019)

Power of performed test with alpha = 0.0500: 0.9984

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	95.5094	4.4906	0.6922	3.9328	(+inf)
2	72.5089	-6.8622	-1.0578	-1.5803	-3.1519

Table A.4 (cont.)

3	56.2587	-0.4169	-0.0643	-0.0824	-0.0674
4	44.8110	-2.3139	-0.3567	-0.4158	-0.3497
5	36.7630	6.3398	0.9772	1.2136	1.3889
6	31.1133	2.4041	0.3706	0.5146	0.4400
7	17.9074	-2.7400	-0.4223	-4.1085	(+inf)

Influence Diagnostics:

Row	**Cook'sDist**	**Leverage**	**DFFITS**
1	120.9552	0.9690	(+inf)
2	0.7692	0.5520	-3.4984
3	0.0011	0.3924	-0.0541
4	0.0155	0.2642	-0.2095
5	0.1997	0.3516	1.0228
6	0.0615	0.4814	0.4240
7	395.1251	0.9894	(+inf)

95% Confidence:

Row	**Predicted**	**Regr. 5%**	**Regr. 95%**	**Pop. 5%**	**Pop. 95%**
1	95.5094	75.1855	115.8332	66.5384	124.4804
2	72.5089	57.1699	87.8479	46.7884	98.2295
3	56.2587	43.3258	69.1916	31.8964	80.6210
4	44.8110	34.1996	55.4223	21.5976	68.0244
5	36.7630	24.5204	49.0057	12.7600	60.7661
6	31.1133	16.7883	45.4384	5.9843	56.2424
7	17.9074	-2.6293	38.4441	-11.2134	47.0282

Table A.5 Regression analysis of the CHO cell BAA DNA repair kinetics data.

Nonlinear Regression

[Variables]
x = col(7)
y = col(8)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 149.841}}
b = xwtr(x,y-min(y),.5)/4 "Auto {{previous: -3.2507}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: 0.422673}}
y0 = min(y) "Auto {{previous: 20.6092}}
[Equation]
f= y0+a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=0.000100
stepsize=100
iterations=100

R = 0.99476894 Rsqr = 0.98956524 Adj Rsqr = 0.97913047

Standard Error of Estimate = 3.8487

	Coefficient	**Std. Error**	**t**	**P**
a	149.8415	112.8701	1.3276	0.2763
b	-3.2507	1.5453	-2.1036	0.1261
x0	0.4227	4.7969	0.0881	0.9353
y0	20.6092	3.9340	5.2388	0.0135

Analysis of Variance:

	DF	**SS**	**MS**	**F**	**P**
Regression	3	4214.1103	1404.7034	94.8335	0.0018
Residual	3	44.4369	14.8123		
Total	6	4258.5472	709.7579		

PRESS = 4800.4290

Durbin-Watson Statistic = 3.0233

Normality Test: K-S Statistic = 0.2042 Significance Level = 0.8975

Constant Variance Test: Passed (P = 0.7810)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	**Predicted**	**Residual**	**Std. Res.**	**Stud. Res.**	**Stud. Del. Res.**
1	100.3939	-0.3939	-0.1024	-0.3131	-0.2599
2	88.8944	1.9699	0.5118	0.6455	0.5680

Table A.5 (cont.)

3	77.7016	-2.5082	-0.6517	-0.8642	-0.8142
4	67.2933	-1.4512	-0.3771	-0.4547	-0.3848
5	58.0172	4.9863	1.2956	1.5756	3.0976
6	50.0581	-2.6713	-0.6941	-1.2067	-1.3735
7	20.7153	0.0686	0.0178	0.5613	0.4844

Influence Diagnostics:

Row	**Cook'sDist**	**Leverage**	**DFFITS**
1	0.2047	0.8931	-0.7512
2	0.0615	0.3713	0.4365
3	0.1416	0.4313	-0.7090
4	0.0235	0.3123	-0.2593
5	0.2973	0.3239	2.1438
6	0.7363	0.6692	-1.9533
7	78.1255	0.9990	15.2571

95% Confidence:

Row	**Predicted**	**Regr. 5%**	**Regr. 95%**	**Pop. 5%**	**Pop. 95%**
1	100.3939	88.8189	111.9690	83.5416	117.2462
2	88.8944	81.4312	96.3577	74.5516	103.2373
3	77.7016	69.6580	85.7453	63.0484	92.3549
4	67.2933	60.4486	74.1381	53.2624	81.3243
5	58.0172	51.0469	64.9875	43.9246	72.1098
6	50.0581	40.0388	60.0774	34.2339	65.8822
7	20.7153	8.4733	32.9573	3.3981	38.0324

Table A.6 Regression analysis of the CHO cell IAA DNA repair kinetics data.

Nonlinear Regression

[Variables]
x = col(4)
y = col(5)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
[Parameters]
a = max(y)-min(y) "Auto {{previous: 2172.73}}
b = xwtr(x,y-min(y),.5)/4 "Auto {{previous: -1.51751}}
x0 = x50(x,y-min(y),.5) "Auto {{previous: -5.0206}}
y0 = min(y) "Auto {{previous: 20.8428}}
[Equation]
f= y0+a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=0.000100
stepsize=100
iterations=100

R = 0.97792049 Rsqr = 0.95632848 Adj Rsqr = 0.93995167

Standard Error of Estimate = 6.0033

	Coefficient	Std. Error	t	P
a	2172.7309	51774.0889	0.0420	0.9676
b	-1.5175	0.9132	-1.6617	0.1351
x0	-5.0206	40.6832	-0.1234	0.9048
y0	20.8428	5.1441	4.0517	0.0037

Analysis of Variance:

	DF	SS	MS	F	P
Regression	3	6313.5262	2104.5087	58.3953	<0.0001
Residual	8	288.3123	36.0390		
Total	11	6601.8385	600.1671		

PRESS = 2080.1444

Durbin-Watson Statistic = 2.3989

Normality Test: K-S Statistic = 0.1353 Significance Level = 0.9705

Constant Variance Test: Passed (P = 0.7831)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	97.5022	2.4978	0.4161	0.1345	0.1260
2	76.5349	-6.1527	-1.0249	-0.3633	-0.3427

Table A.6 (cont.)

3	61.1921	8.5353	1.4218	0.6993	0.6751
4	50.0178	-6.6645	-1.1102	-0.9525	-0.9462
5	41.9075	-1.7635	-0.2938	-1.2842	-1.3482
6	36.0358	0.1806	0.0301	0.0379	0.0355
7	31.7925	-2.0864	-0.3475	-0.3833	-0.3619
8	28.7300	0.2411	0.0402	0.0431	0.0403
9	26.5218	5.1213	0.8531	0.9192	0.9091
10	24.9306	5.4875	0.9141	1.0027	1.0031
11	23.7847	2.2391	0.3730	0.4205	0.3978
12	20.8428	-7.6186	-1.2691	-2.4618	-4.6765

Influence Diagnostics:

Row	**Cook'sDist**	**Leverage**	**DFFITS**
1	-0.0050	10.5623	(+inf)
2	-0.0371	8.9581	(+inf)
3	-0.1518	5.1335	(+inf)
4	-0.3937	2.3586	(+inf)
5	7.4658	0.9477	-5.7372
6	0.0002	0.3716	0.0273
7	0.0080	0.1779	-0.1684
8	0.0001	0.1315	0.0157
9	0.0340	0.1386	0.3647
10	0.0511	0.1690	0.4523
11	0.0120	0.2134	0.2072
12	4.1859	0.7342	-7.7731

95% Confidence:

Row	**Predicted**	**Regr. 5%**	**Regr. 95%**	**Pop. 5%**	**Pop. 95%**
1	97.5022	52.5112	142.4933	50.4296	144.5749
2	76.5349	35.1011	117.9688	32.8497	120.2202
3	61.1921	29.8265	92.5576	26.9074	95.4767
4	50.0178	28.7574	71.2781	24.6476	75.3880
5	41.9075	28.4311	55.3840	22.5877	61.2274
6	36.0358	27.5972	44.4745	19.8231	52.2486
7	31.7925	25.9532	37.6319	16.7678	46.8172
8	28.7300	23.7101	33.7499	14.0044	43.4556
9	26.5218	21.3679	31.6757	11.7500	41.2936
10	24.9306	19.2399	30.6214	9.9631	39.8982
11	23.7847	17.3895	30.1798	8.5354	39.0340
12	20.8428	8.9805	32.7050	2.6121	39.0734

Table A.7 Two-way Analysis of Variance for DNA repair of CHO cells treated with chloroacetic acid (CAA), bromoacetic acid (BAA) and iodoacetic acid (IAA). Holm-Sidak pairwise comparison.

Two Way Analysis of Variance CHO SCGE DNA Repair Kinetics of CAA, IAA and BAA
Multivariate ANOVA Friday, March 06, 2009, 7:50:01 AM

Data source: Data 1 in HAA DNA Repair Kinetics.SNB

Balanced Design

Dependent Variable: TM response

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
HAA	2	11086.049	5543.024	34.981	<0.001
LHT	5	51001.238	10200.248	64.372	<0.001
HAA x LHT	10	3271.643	327.164	2.065	0.036
Residual	90	14261.198	158.458		
Total	107	79620.127	744.113		

The difference in the mean values among the different levels of HAA is greater than would be expected by chance after allowing for effects of differences in LHT. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of LHT is greater than would be expected by chance after allowing for effects of differences in HAA. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of HAA depends on what level of LHT is present. There is a statistically significant interaction between HAA and LHT. (P = 0.036)

Power of performed test with alpha = 0.0500: for HAA : 1.000
Power of performed test with alpha = 0.0500: for LHT : 1.000
Power of performed test with alpha = 0.0500: for HAA x LHT : 0.521

Least square means for HAA :

Group	**Mean**
CAA	56.768
IAA	49.541
BAA	73.715

Std Err of LS Mean = 2.098

Least square means for LHT :

Group	**Mean**
0.000	100.000
1.000	75.413
2.000	57.060
3.000	46.015
4.000	45.916
5.000	35.643

Std Err of LS Mean = 2.967

Least square means for HAA x LHT :

Group	**Mean**
CAA x 0.000	100.000

Table A.7 (cont.)

CAA x 1.000	65.647
CAA x 2.000	55.842
CAA x 3.000	42.497
CAA x 4.000	43.103
CAA x 5.000	33.517
IAA x 0.000	100.000
IAA x 1.000	69.727
IAA x 2.000	40.144
IAA x 3.000	29.706
IAA x 4.000	31.643
IAA x 5.000	26.024
BAA x 0.000	100.000
BAA x 1.000	90.864
BAA x 2.000	75.193
BAA x 3.000	65.842
BAA x 4.000	63.004
BAA x 5.000	47.387

Std Err of LS Mean = 5.139

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: **HAA**

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
BAA vs. IAA	24.174	8.148	2.022E-012	0.017	Yes
BAA vs. CAA	16.947	5.712	0.000000142	0.025	Yes
CAA vs. IAA	7.227	2.436	0.0168	0.050	Yes

Comparisons for factor: **LHT**

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
0.000 vs. 5.000	64.357	15.338	7.646E-027	0.003	Yes
0.000 vs. 4.000	54.084	12.889	3.758E-022	0.004	Yes
0.000 vs. 3.000	53.985	12.866	4.183E-022	0.004	Yes
0.000 vs. 2.000	42.940	10.234	9.356E-017	0.004	Yes
1.000 vs. 5.000	39.770	9.478	3.478E-015	0.005	Yes
1.000 vs. 4.000	29.496	7.030	0.000000000387	0.005	Yes
1.000 vs. 3.000	29.398	7.006	0.000000000431	0.006	Yes
0.000 vs. 1.000	24.587	5.860	0.0000000751	0.006	Yes
2.000 vs. 5.000	21.417	5.104	0.00000184	0.007	Yes
1.000 vs. 2.000	18.353	4.374	0.0000327	0.009	Yes
2.000 vs. 4.000	11.143	2.656	0.00936	0.010	Yes
2.000 vs. 3.000	11.045	2.632	0.00998	0.013	Yes
3.000 vs. 5.000	10.372	2.472	0.0153	0.017	Yes
4.000 vs. 5.000	10.274	2.448	0.0163	0.025	Yes
3.000 vs. 4.000	0.0986	0.0235	0.981	0.050	No

Comparisons for factor: **LHT within CAA**

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
0.000 vs. 5.000	66.483	9.148	0.000	0.003	Yes
0.000 vs. 3.000	57.503	7.912	0.000	0.004	Yes

Table A.7 (cont.)

0.000 vs. 4.000	56.897	7.829	0.000	0.004	Yes
0.000 vs. 2.000	44.158	6.076	0.000	0.004	Yes
0.000 vs. 1.000	34.353	4.727	0.000	0.005	Yes
1.000 vs. 5.000	32.129	4.421	0.000	0.005	Yes
1.000 vs. 3.000	23.150	3.185	0.002	0.006	Yes
1.000 vs. 4.000	22.544	3.102	0.003	0.006	Yes
2.000 vs. 5.000	22.324	3.072	0.003	0.007	Yes
2.000 vs. 3.000	13.345	1.836	0.070	0.009	No
2.000 vs. 4.000	12.739	1.753	0.083	0.010	No
1.000 vs. 2.000	9.805	1.349	0.181	0.013	No
4.000 vs. 5.000	9.585	1.319	0.191	0.017	No
3.000 vs. 5.000	8.980	1.236	0.220	0.025	No
4.000 vs. 3.000	0.606	0.0834	0.934	0.050	No

Comparisons for factor: **LHT within IAA**

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
0.000 vs. 5.000	73.976	10.179	0.000	0.003	Yes
0.000 vs. 3.000	70.294	9.672	0.000	0.004	Yes
0.000 vs. 4.000	68.357	9.406	0.000	0.004	Yes
0.000 vs. 2.000	59.856	8.236	0.000	0.004	Yes
1.000 vs. 5.000	43.704	6.013	0.000	0.005	Yes
1.000 vs. 3.000	40.021	5.507	0.000	0.005	Yes
1.000 vs. 4.000	38.084	5.240	0.000	0.006	Yes
0.000 vs. 1.000	30.273	4.165	0.000	0.006	Yes
1.000 vs. 2.000	29.583	4.071	0.000	0.007	Yes
2.000 vs. 5.000	14.120	1.943	0.055	0.009	No
2.000 vs. 3.000	10.438	1.436	0.154	0.010	No
2.000 vs. 4.000	8.501	1.170	0.245	0.013	No
4.000 vs. 5.000	5.619	0.773	0.441	0.017	No
3.000 vs. 5.000	3.682	0.507	0.614	0.025	No
4.000 vs. 3.000	1.937	0.267	0.790	0.050	No

Comparisons for factor: **LHT within BAA**

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
0.000 vs. 5.000	52.613	7.239	0.000	0.003	Yes
1.000 vs. 5.000	43.478	5.982	0.000	0.004	Yes
0.000 vs. 4.000	36.996	5.091	0.000	0.004	Yes
0.000 vs. 3.000	34.158	4.700	0.000	0.004	Yes
1.000 vs. 4.000	27.861	3.834	0.000	0.005	Yes
2.000 vs. 5.000	27.807	3.826	0.000	0.005	Yes
1.000 vs. 3.000	25.022	3.443	0.001	0.006	Yes
0.000 vs. 2.000	24.807	3.413	0.001	0.006	Yes
3.000 vs. 5.000	18.455	2.539	0.013	0.007	No
1.000 vs. 2.000	15.671	2.156	0.034	0.009	No
4.000 vs. 5.000	15.617	2.149	0.034	0.010	No
2.000 vs. 4.000	12.190	1.677	0.097	0.013	No
2.000 vs. 3.000	9.351	1.287	0.201	0.017	No
0.000 vs. 1.000	9.136	1.257	0.212	0.025	No
3.000 vs. 4.000	2.839	0.391	0.697	0.050	No

Table A.7 (cont.)

Comparisons for factor: **HAA within 0**

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
CAA vs. IAA	1.421E-013	1.955E-014	1.000	0.017	No
CAA vs. BAA	4.263E-014	5.866E-015	1.000	0.025	No
BAA vs. IAA	9.948E-014	1.369E-014	1.000	0.050	No

Comparisons for factor: **HAA within 1**

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
BAA vs. CAA	25.218	3.470	0.001	0.017	Yes
BAA vs. IAA	21.137	2.908	0.005	0.025	Yes
IAA vs. CAA	4.081	0.561	0.576	0.050	No

Comparisons for factor: **HAA within 2**

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
BAA vs. IAA	35.049	4.823	0.000	0.017	Yes
BAA vs. CAA	19.352	2.663	0.009	0.025	Yes
CAA vs. IAA	15.698	2.160	0.033	0.050	Yes

Comparisons for factor: **HAA within 3**

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
BAA vs. IAA	36.136	4.972	0.000	0.017	Yes
BAA vs. CAA	23.345	3.212	0.002	0.025	Yes
CAA vs. IAA	12.791	1.760	0.082	0.050	No

Comparisons for factor: **HAA within 4**

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
BAA vs. IAA	31.360	4.315	0.000	0.017	Yes
BAA vs. CAA	19.901	2.738	0.007	0.025	Yes
CAA vs. IAA	11.460	1.577	0.118	0.050	No

Comparisons for factor: **HAA within 5**

Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significant?**
BAA vs. IAA	21.363	2.939	0.004	0.017	Yes
BAA vs. CAA	13.869	1.908	0.060	0.025	No
CAA vs. IAA	7.494	1.031	0.305	0.050	No

Table A.8 Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with chloroacetic acid without and with 1, 2, 3, 4, 5 and 24 hour liquid-holding recovery.

LH_0h	LH_1h	LH_2h	LH_3h	LH_4h	LH_5h	LH_24h
1.89816	1.77412	1.16929	0.482543	0.350030	0	0
7.39122	2.59485	1.46925	0.529053	0.353541	0	0
8.00026	3.34571	1.62063	0.607267	0.641992	0	0
14.3215	3.35549	1.62280	0.779692	0.838712	0	0
15.3750	3.39348	1.68511	1.26777	1.70883	0.148729	0
16.0592	3.95373	1.92082	1.28495	1.85396	0.196121	0
18.7389	4.26208	2.03675	1.28776	1.98630	0.289881	0
19.2952	4.41890	2.64310	1.49251	2.69055	0.384716	0
21.0806	4.56261	3.07576	1.51283	2.78892	0.414667	0
22.5384	4.58761	3.28975	1.70365	3.02022	0.448623	0
22.9453	4.65933	3.31564	1.75470	3.12454	0.478664	0
25.9863	4.76807	3.33981	1.77765	3.28541	0.488387	0
27.1813	5.11083	3.47893	1.82869	3.38324	0.531871	0
27.5630	5.14193	4.17077	1.83651	3.50514	0.540641	0
28.0050	5.21619	4.35420	1.84580	3.64082	0.576870	0
29.8275	5.45530	4.90067	1.90539	3.89074	0.603571	0
31.2193	5.51990	5.08070	1.92470	4.06167	0.614740	0
32.2386	6.00556	5.32571	2.00791	4.08896	0.615207	0
32.8749	6.15730	5.41289	2.10000	4.08972	0.630892	0
32.9964	7.80491	5.51314	2.13922	4.15777	0.700391	0.0808468
33.7740	7.94536	6.01529	2.27459	4.16729	0.710434	0.138006
33.9724	8.31093	6.16920	2.79064	4.44365	0.736958	0.154044
34.1381	8.85729	6.52334	3.00173	4.45763	0.750094	0.160460
35.9517	10.3555	7.38441	3.03040	4.52910	0.759171	0.239990
35.9907	10.6110	7.50076	3.06186	4.72121	0.792267	0.256281
36.7876	10.6532	8.15320	3.21584	5.07226	0.819058	0.266110
37.1009	10.9274	8.55583	3.33348	5.12261	0.942236	0.304608
37.2197	11.1704	8.57860	3.46350	5.15331	1.00684	0.308193
37.4286	11.4310	8.77739	3.61654	5.17934	1.01790	0.314668
37.4890	11.5695	8.81375	3.78041	5.18826	1.13393	0.319999
37.5309	11.6917	8.81682	4.26299	5.23506	1.15095	0.321096
37.7241	11.9239	8.91645	4.34719	5.41999	1.22039	0.340884
38.0050	12.0145	9.22742	4.47698	5.57999	1.22538	0.350860
38.5558	12.9912	9.47527	4.67889	5.83256	1.40237	0.357560
38.7435	13.0612	9.65651	4.69599	5.95190	1.52265	0.371352
38.7927	13.7958	9.79066	4.74216	6.06289	1.52789	0.381534
39.1583	13.8053	9.82831	4.89648	6.07137	1.53866	0.381579
39.3033	13.8135	9.83719	4.96933	6.11791	1.54519	0.393645
39.4289	14.4146	9.89110	5.31258	6.18442	1.61218	0.413302
39.5685	14.5021	10.2702	5.44492	6.33499	1.64745	0.416770
40.0943	14.8086	10.5414	5.51565	6.34564	1.68684	0.423316
41.1504	15.0653	10.6251	5.75446	6.44886	1.69394	0.426014
41.2008	15.2647	11.0245	6.08772	6.62914	1.71788	0.443669
41.4843	15.4527	11.2362	6.11111	7.34096	1.72778	0.448801

Table A.8 (cont.)

41.5773	15.6326	11.5932	6.15696	7.44516	1.75717	0.464367
41.8024	16.0957	11.6632	6.15888	7.58022	1.75831	0.469015
41.8853	16.0959	11.7837	6.16124	7.86144	1.76557	0.471124
42.7538	16.3362	11.8102	6.25887	7.88376	1.80173	0.472750
43.5761	16.4091	11.9174	6.32208	7.89158	1.97704	0.486529
43.7446	17.0214	12.2850	6.46884	8.46538	2.05753	0.490868
44.0778	17.3285	12.4460	6.64745	8.71526	2.15650	0.498766
44.3715	17.7385	12.8290	6.70308	8.86905	2.18418	0.519064
44.5412	17.7458	12.8527	6.78007	8.91000	2.26551	0.545962
44.6001	17.9831	12.8990	6.90868	9.05967	2.30724	0.547500
44.6705	18.1465	12.9304	7.14435	9.22028	2.35108	0.558472
44.8967	18.3341	13.4411	7.15651	9.26245	2.35883	0.562203
45.0677	18.7882	13.5392	7.24044	9.36710	2.41625	0.568106
45.1431	19.1552	13.5874	7.34831	9.65808	2.54205	0.570116
45.3590	19.5629	13.7845	7.40378	9.72747	2.58395	0.588025
45.3693	19.8115	14.1012	7.40834	10.2318	2.59986	0.594232
45.5849	20.7844	14.2238	7.51876	10.2652	2.61510	0.594853
46.2216	21.1039	14.2949	7.55922	10.2807	2.65567	0.596218
46.4027	21.5205	14.5445	7.74696	10.3095	2.72047	0.640699
46.4252	22.2985	14.7532	7.92849	10.5129	2.82028	0.646924
46.8002	23.3823	14.9065	7.95708	10.9494	2.85123	0.649090
47.8239	24.0946	14.9432	8.01726	10.9868	2.91446	0.659185
47.8806	24.1207	14.9657	8.19154	11.1901	2.91677	0.679834
47.9484	25.1399	15.0406	8.21362	11.3067	3.01478	0.681980
48.0237	25.8794	15.7120	8.25374	11.5203	3.06245	0.702359
48.2850	25.9305	15.8329	8.28889	11.5948	3.07344	0.708176
48.5354	26.7125	15.8480	8.40720	11.7167	3.38766	0.709348
49.3044	26.7225	16.0067	8.46684	11.9505	3.54013	0.720234
49.6102	26.9324	16.8826	8.67575	12.1361	3.59080	0.722307
49.8943	27.5139	17.2734	8.75467	12.2843	3.60676	0.727039
51.1183	27.6363	17.5234	8.80430	12.3702	3.62238	0.745446
51.3893	28.3406	17.8296	9.07924	12.4311	3.69019	0.760113
51.9270	28.4564	18.2510	9.17667	12.5170	3.72629	0.770382
52.5346	29.0681	18.4494	9.25706	12.5536	3.77720	0.771286
52.9054	29.2274	18.5267	9.42419	12.6047	4.04968	0.781912
53.0287	29.5457	18.9170	9.57692	12.7513	4.07496	0.789531
53.6418	30.0179	19.0773	9.79433	12.7707	4.13724	0.789655
53.9699	30.0861	19.2755	9.95698	12.8348	4.36412	0.826512
54.2048	30.2376	19.4095	10.3194	13.1344	4.44183	0.826905
54.2759	30.7207	20.3666	10.4319	13.3919	4.44294	0.850715
54.4144	30.8079	20.8902	10.4416	13.4940	4.45117	0.871834
54.4763	30.8369	21.1579	10.4565	13.6837	4.50930	0.873851
54.6678	30.9512	21.5969	10.6277	13.7041	4.61657	0.907581
54.6983	31.0758	21.6521	10.6987	13.8211	4.62599	0.937309
54.8863	31.3732	21.9781	10.7154	13.8616	4.79998	0.983015
55.4208	31.5942	22.4423	10.8061	14.0998	4.85177	0.983981
55.9893	31.7248	22.4442	10.8349	14.3275	4.91408	0.99189
56.0106	31.7383	22.4499	10.9538	14.7054	4.96918	1.00992

Table A.8 (cont.)

56.4772	31.8711	22.7809	10.9636	15.1455	4.97124	1.02686
56.6316	32.2336	22.9808	11.0214	15.2125	5.01713	1.03752
56.7080	32.7337	23.2279	11.1355	15.4233	5.13521	1.03785
56.8418	33.0218	23.4042	11.2826	15.6909	5.14173	1.06812
57.1074	33.0817	23.4863	11.8629	15.8304	5.19782	1.07062
57.1991	33.2297	24.2411	12.1460	15.8494	5.42776	1.07507
57.2233	33.4164	24.7252	12.2308	16.0950	5.44417	1.07923
57.5054	33.4769	25.0337	12.2479	16.2661	5.73717	1.10754
57.8425	33.4950	25.0814	12.4356	16.3305	5.90226	1.10895
57.9123	33.5998	25.3364	12.5451	16.5587	5.90493	1.11299
58.4381	33.8048	25.8968	12.6782	16.8633	6.11333	1.12745
59.1624	33.8385	26.0070	12.7307	16.8673	6.22841	1.15067
59.1912	33.8575	26.1277	13.4415	17.0508	6.23411	1.16601
59.2117	33.8687	26.6492	13.4428	17.1184	6.32961	1.17061
59.7406	34.0069	26.7238	13.5895	17.3953	6.77653	1.20620
59.7601	34.3206	27.1719	13.6198	17.5146	6.98630	1.21527
60.3936	34.4319	27.2982	13.8153	17.5919	7.03433	1.23452
60.4289	34.6865	27.4858	14.2361	17.6424	7.08749	1.25067
60.5303	34.9810	28.1111	14.4146	17.7401	7.25115	1.26284
60.9584	34.9929	28.1303	14.7433	17.9507	7.28821	1.27197
61.1963	35.4880	28.3188	15.1534	17.9908	7.35173	1.28150
61.3347	35.5934	28.8220	15.1732	18.1509	7.41179	1.28156
61.7614	35.6941	28.9220	15.3637	18.1684	7.50885	1.31095
61.9165	36.1317	29.0952	15.4150	18.5311	7.58459	1.35160
62.0060	36.1754	29.1700	15.4553	18.6842	7.71378	1.36850
62.1465	36.3512	29.2909	15.9930	18.7141	7.78236	1.36871
62.1996	36.5398	29.3561	16.1811	19.4553	7.79552	1.41674
62.5267	36.6105	29.4571	16.2292	19.5516	7.99974	1.44654
62.6414	36.8366	30.0550	16.2293	19.6179	8.03869	1.46682
62.7677	36.9324	30.2016	16.2437	19.7414	8.07551	1.51980
62.8837	37.0396	30.2352	16.6120	20.1416	8.21676	1.59799
63.3507	37.1507	30.3593	16.9115	20.3958	8.35725	1.60433
63.4483	37.2243	30.3762	17.1388	20.4815	8.39504	1.66802
63.4824	37.4063	30.7503	17.1948	20.7771	8.46295	1.68321
63.5203	37.7167	30.7997	17.5563	20.9831	8.65736	1.71867
63.5534	37.8914	30.9274	17.9019	20.9948	8.70048	1.74663
63.8465	37.9406	31.0861	17.9380	21.1744	8.75706	1.76670
64.0501	38.0923	31.4230	18.2069	21.5116	8.76450	1.79632
64.1817	38.2787	31.5169	18.3361	21.6751	8.86750	1.80421
64.5666	39.2078	31.5701	18.3369	21.9149	9.25295	1.88057
64.7755	39.4304	31.7355	18.4881	22.0514	9.34673	1.88319
64.9193	39.4835	31.7405	18.5094	22.1161	9.42093	1.91744
65.2833	39.5415	31.8006	18.5150	22.1200	9.66672	1.93813
65.4122	39.7488	31.9951	18.5324	22.4679	9.69090	1.95188
65.4267	40.1414	32.2353	18.6880	23.6986	9.85090	1.97305
65.4490	40.1842	32.8060	18.8611	23.9672	10.2934	2.03622
65.5276	40.3493	33.1388	19.0281	24.1544	10.7458	2.12081
65.7769	40.9129	33.3142	19.0490	24.4063	10.7978	2.15059

Table A.8 (cont.)

65.7986	41.0975	33.5751	19.1604	24.4911	10.9413	2.18292
65.8179	41.3890	33.8732	19.1959	24.8429	11.0844	2.20451
65.8795	41.6215	34.1241	19.2270	25.2252	11.1739	2.20904
66.0052	41.9791	34.2013	19.3532	25.4817	11.1968	2.26019
66.0318	42.3914	34.4009	19.9478	25.6872	11.4581	2.26554
66.4653	42.5544	34.5058	20.2047	25.7861	11.7724	2.35333
66.5489	42.5847	35.0071	20.2545	26.0040	11.8490	2.35556
66.7550	42.7408	35.8920	20.5492	26.3381	11.9564	2.38607
67.0405	43.4471	35.8988	20.8246	26.3766	12.2137	2.42031
67.0662	43.4931	35.9284	21.5638	26.4338	12.2545	2.44167
67.2971	43.5215	36.3006	22.4590	26.6305	12.8122	2.46047
67.5533	43.7783	36.3259	22.5031	26.7012	12.9124	2.47967
68.0369	43.8098	36.3483	22.7206	26.7924	12.9288	2.48573
68.1695	43.9632	36.6656	22.7786	26.9461	13.0132	2.56485
68.2711	44.3015	36.8206	22.8772	27.3607	13.0624	2.67199
68.5208	44.4385	37.1515	22.9601	27.4072	13.1244	2.67245
68.6261	44.6922	37.4520	23.1028	27.5070	13.1837	2.78009
68.7324	45.0764	37.4567	23.1164	27.5321	13.9763	2.79452
68.7461	45.0875	37.4734	24.4441	27.5772	14.0264	2.86296
68.7863	45.0932	37.4787	25.0046	27.9648	14.0519	2.87065
68.9943	45.3377	37.6077	25.1087	28.1116	14.1325	2.90980
69.0488	45.4005	37.7939	25.1551	28.2300	14.4841	2.94761
69.1251	45.4868	37.9778	25.6249	28.3775	14.5737	2.95299
69.2787	45.4939	38.1209	25.8334	28.5060	14.6134	2.98804
69.5482	46.0861	38.1410	26.1527	28.5521	14.7436	3.00695
69.8598	46.2573	38.3156	26.1645	28.5581	14.9133	3.13932
69.8795	46.3503	38.7817	26.1811	28.5774	15.0953	3.16561
69.8805	46.3651	39.3053	26.3955	28.6196	15.2539	3.20850
69.9315	46.6603	39.8885	26.6361	28.7613	15.8866	3.21365
70.3967	47.1017	39.8945	27.2032	28.9332	16.1117	3.24208
70.4060	47.1741	39.9972	27.3127	29.4694	16.1298	3.30670
70.6888	47.4222	40.5332	27.3728	29.6541	16.3337	3.38491
70.8514	47.8018	41.0549	27.8607	29.9574	16.5740	3.40661
70.9906	47.8343	41.2904	28.1110	30.3791	16.6295	3.41881
71.0322	47.8864	41.4207	28.2148	30.4197	17.2086	3.42789
71.2235	48.1516	41.8465	28.3230	30.4676	17.3502	3.50654
71.3834	48.1897	42.1042	28.8264	30.5382	17.5145	3.52099
71.4157	48.2020	42.3182	29.2484	30.6876	17.8965	3.52923
71.4751	48.2726	42.5520	29.3013	30.9981	17.9123	3.53259
71.9664	48.7052	42.7158	29.4391	31.2371	18.0219	3.55815
72.0408	48.7169	42.7363	29.4972	31.3370	18.0495	3.56510
72.1169	49.5623	42.7493	30.4112	31.4320	18.3635	3.61193
72.1846	49.8467	42.8791	31.0027	31.9415	18.3801	3.75118
72.3709	49.8847	43.0946	31.0204	32.4503	18.4230	3.78466
72.4642	50.2439	43.1154	31.4540	32.8623	18.7573	3.78856
72.9687	50.3088	43.1739	31.6138	33.4627	18.7919	3.79030
73.0699	50.3192	43.5247	31.9385	33.4979	18.8177	3.82858
73.1178	50.6214	43.6730	32.6793	33.5456	19.3185	3.83966

Table A.8 (cont.)

73.1906	50.6238	44.0493	32.7358	33.8498	19.6769	3.86969
73.2937	50.7831	44.1224	32.8934	34.1343	20.3196	3.91801
73.7330	50.9122	44.1415	33.5773	34.5232	20.6369	3.92312
73.8175	51.2421	45.2970	33.5863	34.6666	20.9039	3.94850
73.8358	51.3716	45.4607	33.7617	35.2948	21.1614	3.98940
73.8802	51.5229	45.4976	34.3360	35.3273	21.2176	4.01784
74.5400	51.8358	45.5969	34.4514	35.4112	21.5798	4.03037
74.6608	52.2023	46.0050	35.4504	35.6387	22.1417	4.03037
74.6753	52.2240	46.4447	35.7638	36.2523	22.2078	4.05095
74.9080	52.3135	46.7951	35.8683	36.6818	22.6356	4.07220
75.0025	52.4082	46.8245	36.0447	36.8123	23.1120	4.10316
75.0067	52.4974	47.0852	36.2536	36.8287	23.4068	4.14425
75.3542	52.9621	47.1283	36.4839	36.8744	24.1093	4.16046
75.5233	53.1154	47.5949	36.5052	37.2649	24.3856	4.20154
75.5740	53.4818	48.3689	37.6834	37.3876	24.8214	4.30792
75.6679	53.5145	48.4340	37.8010	37.6004	25.1054	4.32742
75.7667	53.5227	49.0332	38.1049	37.7627	26.7049	4.49064
76.0405	53.5408	49.2462	38.1674	37.8330	27.7758	4.59134
76.1249	53.7746	49.2777	38.5484	37.8689	27.9195	4.59502
76.2192	53.8699	49.3786	38.6538	37.9554	27.9840	4.63470
76.2863	54.5477	49.5517	38.8870	38.3320	28.2051	4.63533
76.4718	54.7531	49.9610	40.2914	38.4417	28.5494	4.74321
76.4835	54.8839	50.2015	40.4638	38.4528	29.1238	4.78612
76.5848	56.2589	50.3797	40.5633	38.4555	29.1717	4.84615
76.6478	56.3927	50.5276	40.7097	38.5183	29.5400	4.89069
76.7660	56.4115	50.5461	41.0484	38.5343	29.7184	5.09582
76.9729	56.5518	50.5612	41.2592	38.6735	29.8568	5.12540
77.0952	56.5780	50.9760	41.6620	38.7998	30.2314	5.16302
77.0982	56.9554	51.0750	41.6849	39.0109	30.4008	5.20879
77.0991	57.3085	51.4197	41.7486	39.0166	32.2971	5.21097
77.3131	57.3628	51.7648	43.1041	39.1185	32.3922	5.27940
77.5215	57.4039	51.7773	43.5580	39.2384	32.4156	5.33590
77.6165	57.6923	51.8825	43.6382	39.2506	32.6815	5.34577
77.6320	58.0432	52.6871	43.6455	39.6778	32.7782	5.37263
77.6755	58.0655	52.9705	43.8434	39.9389	33.0810	5.40667
77.7665	58.6723	53.0168	44.1652	40.0847	33.0868	5.44062
78.0162	59.5949	53.8129	44.2848	40.2242	33.2865	5.46017
78.0416	60.0784	53.8412	44.4309	40.2626	34.2763	5.49140
78.0873	60.5033	54.1744	44.7214	40.8374	34.3262	5.51818
78.1452	60.8341	54.5266	45.0921	41.4938	34.5558	5.59912
78.3773	61.0240	54.6407	45.7910	41.8169	34.5627	5.67074
78.3891	61.1997	54.6794	46.1040	42.3636	34.5906	5.85374
78.4777	61.4793	55.1572	46.4831	42.5351	34.6482	5.86007
78.4959	61.5517	55.4104	46.4903	42.6392	34.9587	5.94080
78.5435	61.6072	55.5023	46.8901	42.7282	35.9118	5.96894
78.6766	61.8707	55.6151	46.9145	42.9645	35.9668	6.00709
78.6976	61.9286	55.6294	47.3870	43.2914	36.6474	6.05015
78.7841	62.0916	55.6320	47.8488	43.3943	36.8200	6.13273

Table A.8 (cont.)

78.8600	62.7945	55.6640	48.2302	43.4280	37.1967	6.15175
79.0939	63.2479	55.7788	48.3234	43.6158	37.2268	6.15540
79.6473	63.2510	56.0324	48.3264	43.9333	37.7066	6.17994
79.6873	63.7026	56.0603	48.3796	43.9565	37.7921	6.33010
79.9799	63.8257	56.1254	48.5697	44.1826	38.0049	6.39699
80.1317	63.9884	56.7201	49.0392	44.3099	38.3963	6.40890
80.2049	64.0294	56.7307	49.2114	44.4553	39.2665	6.45471
80.4886	64.5289	56.7358	49.3486	44.5539	39.5267	6.45972
80.8087	64.6686	57.0334	49.4432	44.8650	40.0749	6.49373
80.8346	64.7020	57.4683	50.3897	44.8772	40.1753	6.60618
80.8924	65.2897	57.9163	50.4459	46.0745	40.7107	6.70553
80.9818	65.5157	57.9361	50.6498	46.1065	40.8433	6.73492
81.0334	66.1214	58.1721	50.8564	46.2416	41.3021	6.78849
81.0693	66.3170	58.2534	50.9682	46.3263	41.5878	6.84975
81.2523	66.3789	58.4856	51.3548	46.4649	41.6996	6.87091
81.3633	67.0465	58.8079	51.3734	46.7627	41.8136	6.88238
81.5749	67.0576	58.8875	51.5766	46.9020	41.8981	6.99425
81.5988	67.2772	58.9408	51.7930	46.9503	42.3553	7.02957
82.1061	67.4535	59.2072	51.8419	47.2941	42.6584	7.12467
82.1669	67.5113	59.4822	51.9596	47.3291	42.9795	7.14795
82.1780	67.7781	59.9154	52.0025	47.8108	44.2724	7.47934
82.2562	67.7902	60.3092	52.3394	48.4717	44.7142	7.57612
82.3107	69.6118	60.3941	52.5059	48.7726	45.3310	7.63380
82.4037	69.8032	61.0509	52.8066	48.8073	45.6046	7.67688
82.4696	70.0140	61.2655	53.4224	48.9842	46.1532	7.70104
82.4986	70.1887	61.2892	53.5041	49.3691	46.7454	7.72406
82.7153	70.4670	61.6331	53.6365	49.5068	47.8487	7.80497
82.9325	70.5708	62.0998	53.9060	49.5834	48.0944	7.82346
82.9692	70.5783	62.4167	54.1294	49.6399	50.4413	7.86882
83.0994	70.9181	63.0612	54.3133	49.8853	50.6080	7.92955
83.3118	71.0882	63.4231	54.9186	50.8813	50.8168	7.96036
83.3425	71.3808	63.6767	55.0789	51.2195	52.1457	8.01677
83.4245	71.3969	63.7868	55.8273	52.6798	53.1795	8.04830
83.4914	71.9836	64.1853	56.0914	54.0095	53.3503	8.50333
83.5686	72.4192	64.7631	56.5232	54.0822	53.5211	8.55134
83.5754	72.6086	65.2533	57.2189	54.3403	55.2030	8.69077
83.7845	72.6562	65.4510	57.7044	54.5578	56.7411	8.75986
83.8238	73.3625	65.5143	59.0303	54.6476	57.1240	9.06653
83.9344	73.5007	66.1267	59.1145	55.4654	57.7208	9.20712
84.0731	73.5652	66.4863	60.8307	56.2124	58.0728	9.22870
84.3391	74.6572	66.6803	61.0424	56.3218	58.2025	9.55736
84.3752	76.1434	66.7403	61.9892	56.3701	58.4242	9.57864
84.3782	76.7683	66.8813	62.8933	56.8899	58.6146	9.67728
84.4854	76.9912	67.2381	63.7757	58.2442	58.8480	9.71964
84.5637	77.3347	67.8000	64.5516	58.2828	61.7688	9.75133
85.0535	77.8959	68.3788	64.9791	58.7276	62.2380	9.77275
85.1598	78.4472	68.6121	65.4592	60.3931	62.2580	9.81424
85.2469	78.6101	68.9936	68.2160	60.9010	62.8396	9.83628

Table A.8 (cont.)

85.3449	79.2764	70.0923	68.5319	61.5459	65.2431	9.87821
85.4557	79.6210	72.5319	69.1048	63.5871	66.2725	10.2282
85.6731	80.2971	72.6986	69.8794	64.2027	66.3652	10.6903
85.6807	80.4100	73.2221	70.1614	64.9107	67.8455	10.8451
85.8216	80.5047	73.8006	70.3688	65.8702	68.9266	10.9429
86.2697	81.0870	75.1063	70.6155	68.6585	70.0337	10.9855
86.2791	81.1323	75.7316	70.6788	70.8078	70.4059	10.9981
86.2917	82.4307	76.3749	70.7257	72.5679	70.5599	11.0136
86.3886	83.7925	76.9179	71.0776	74.3719	70.9934	11.0719
86.4182	85.1371	78.3113	72.7913	74.4413	75.5793	11.1179
86.5326	85.2350	78.5276	73.6660	81.4663	77.8335	11.2573
86.7809	85.9876	79.6538	74.5566	81.4724	78.7976	11.3302
86.9058	87.1466	86.8656	78.4499	81.5506	80.7001	11.7910
87.2931	88.6443	89.5709	83.4172	81.7990	80.9494	11.7918
87.4354	88.9822	90.2665	88.8817	84.2838	84.6904	12.4074
87.5262	93.5679	91.5425	89.3336	85.5011	90.1872	12.6249
87.6504						12.7054
87.8650						12.9336
88.1693						12.9972
88.2779						13.3760
88.3035						13.4564
88.3230						13.7047
88.3251						13.8943
88.3579						14.7948
88.5540						14.8290
88.5684						15.0182
88.9346						15.9526
89.5730						16.5896
89.6015						17.7509
89.6155						18.0214
89.6878						18.2497
89.6939						18.6275
89.7878						18.7202
89.9481						19.2617
90.2357						19.7831
90.3589						20.2298
90.5157						20.2817
90.6805						20.5614
90.8555						20.9337
90.8839						22.2604
91.0796						22.5333
91.1337						23.8128
91.2539						23.8880
91.6053						24.1199
91.6773						24.1782
91.7474						24.2760
92.0823						24.4222
92.4171						24.8946

Table A.8 (cont.)

92.5441	25.8861
92.7075	28.9321
92.7191	29.7889
92.7481	30.1330
92.7926	30.9538
92.9197	36.5969
92.9393	37.0907
93.0586	37.7085
93.5973	41.3614
93.7435	49.7953
93.7621	54.3988
93.7636	57.7578
93.8237	57.8155
94.4617	59.4862
94.7185	68.4189
94.7746	70.8857
95.4379	73.4599
98.3215	75.9515

Table A.9 Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with bromoacetic acid without and with 1, 2, 3, 4, 5 and 24 hour liquid-holding recovery.

LH_0h	LH_1h	LH_2h	LH_3h	LH_4h	LH_5h	LH_24h
1.09666	1.29940	1.66620	0.732319	0.453507	0.294216	0.224926
1.42283	2.65633	2.62503	0.955142	0.600620	0.640939	0.286862
3.12683	5.49852	3.12556	0.961374	0.772081	0.768654	0.293668
4.22903	8.21766	3.14566	1.03240	1.03703	0.774926	0.315953
5.76477	8.40428	3.24547	1.07134	1.35429	0.807261	0.333389
5.97487	9.95728	3.56065	1.48010	1.42242	0.890267	0.479498
7.41226	10.0694	3.60688	1.54529	1.42911	1.01836	0.524173
7.91857	10.6771	4.40058	1.68078	2.00171	1.05843	0.563811
9.48337	11.3689	4.65385	1.84901	2.08350	1.07983	0.565986
10.0933	11.5059	4.88941	1.96864	2.14517	1.08551	0.613075
10.2858	12.4314	5.04619	2.02544	2.16871	1.13672	0.628614
10.5264	12.6824	6.31435	2.13685	2.19426	1.21549	0.649479
12.6905	13.2133	6.64394	2.19717	2.20665	1.28741	0.688622
12.9485	13.5112	6.82940	2.24385	2.23967	1.41307	0.700107
13.4350	13.5283	7.72659	2.42345	2.26058	1.55658	0.734889
13.8821	13.9412	8.14138	2.74722	2.37336	1.59376	0.741270
15.0287	14.0735	8.16478	3.23905	2.38978	1.60425	0.830427
15.1438	14.8618	8.25480	3.28995	2.60566	1.60720	0.855867
15.5046	15.1819	9.18469	3.35317	2.79775	1.67389	0.898520
15.6487	15.2011	9.19701	3.81939	2.81202	1.69563	0.906166
16.1897	15.2100	9.30631	3.86799	3.07896	1.79014	0.916526
18.4253	15.8154	9.49591	3.87404	3.22815	1.86041	0.921815
19.6120	17.3136	9.70040	4.26277	3.37166	1.86926	0.949078
19.7484	17.5227	10.2263	4.47075	3.45478	1.87851	0.955428
20.3202	17.8665	10.2573	4.54499	3.51138	2.01292	0.998819
20.5430	18.0539	10.3871	4.68267	3.63834	2.17596	1.00302
20.7742	18.3124	10.9167	5.41589	3.66680	2.19733	1.01031
20.8377	18.5113	11.1532	5.56586	4.50489	2.34170	1.05416
20.8783	18.5954	11.3443	5.58413	4.59940	2.50257	1.10489
21.0544	19.1456	11.6040	6.18863	5.17258	2.51935	1.16688
21.0773	19.1874	12.1711	6.39282	5.69084	2.60718	1.19321
23.1099	19.5522	12.7959	6.89663	5.76056	2.76528	1.21668
23.1359	19.5821	12.7991	7.20113	5.77570	2.85524	1.22568
23.7171	19.8336	13.3610	7.21536	5.81127	2.94822	1.24625
24.0415	19.8813	14.3746	7.38543	5.89151	3.39613	1.25227
24.1958	19.8831	14.4939	7.75977	6.27036	3.60581	1.27137
24.2824	20.2600	14.7116	8.12358	6.59591	3.75969	1.28005
24.6218	20.3891	14.7332	8.22596	6.63519	3.90089	1.35811
24.7165	20.7605	15.4598	8.26189	6.68194	3.92280	1.37011
24.9854	21.0527	15.6444	8.34262	7.16090	4.10494	1.42648
25.3057	21.2175	15.9216	8.53496	7.17994	4.14354	1.42793
25.3119	21.3454	16.3211	8.53776	7.30625	4.17513	1.43377
25.5217	22.1687	16.3708	9.22511	7.44569	4.79986	1.44220
25.5533	22.6140	16.3824	9.25548	7.49363	5.01713	1.47707

Table A.9 (cont.)

25.5651	22.8048	16.6612	9.39302	8.11156	5.48318	1.49729
25.6547	22.9340	17.0190	9.52477	8.33115	5.53361	1.53349
25.8676	23.1029	17.2345	9.63285	8.58649	5.66361	1.57145
25.9248	23.1664	17.3484	9.68705	8.70622	5.67012	1.62404
26.2087	23.5484	17.6263	10.3178	9.85394	5.74451	1.63189
26.4943	23.6612	17.7025	11.1356	10.1132	5.91318	1.64619
26.5633	23.9088	17.7072	11.1666	10.3216	6.07570	1.65756
26.5974	24.5334	18.1108	11.6015	10.4798	6.25979	1.80206
26.9372	24.6189	18.2816	11.6193	10.5578	6.34196	1.84415
26.9880	25.2466	18.8532	11.7263	10.7455	6.50972	1.84986
27.8664	25.4057	18.8969	11.8168	11.6994	6.57001	1.85039
28.2994	25.4387	19.5696	11.8931	11.7151	6.77993	1.85564
28.6864	25.4528	19.6003	12.4079	12.0233	6.84412	1.93654
29.2030	25.5858	19.7108	12.5099	12.0335	7.10327	1.94548
29.2903	25.7238	20.0637	12.7070	12.0863	7.10701	1.97661
29.5874	25.8490	20.1123	13.2210	12.2360	7.12876	1.99077
29.7888	26.2829	20.1591	13.2834	12.2910	7.26396	1.99171
30.1029	26.4888	20.3468	13.3454	12.3428	7.36014	2.02787
30.1664	26.9960	20.4256	13.7689	12.4097	7.69451	2.09390
30.2274	27.0956	20.4858	13.8455	12.6021	7.88887	2.16513
30.3970	27.2096	20.6039	14.0685	13.4104	7.90987	2.16614
30.6921	27.3058	20.6067	14.3347	13.5820	7.94689	2.29880
30.6959	27.5137	20.9914	14.4628	13.7208	8.06642	2.30022
30.8406	27.6446	21.3361	14.8832	14.1709	8.28730	2.30494
30.8513	28.1058	21.3414	14.8912	14.3983	8.32468	2.42938
31.4866	28.3105	21.3449	14.9102	14.5605	8.53095	2.46166
31.8371	28.5600	21.4443	15.8145	14.6766	8.82330	2.50473
32.3907	28.7024	21.4545	16.0798	14.7682	8.89829	2.54696
32.4032	29.6553	21.8250	16.1245	15.0774	8.98401	2.65772
32.4647	29.8391	21.9417	16.3185	15.1473	9.00249	2.73266
32.4778	29.8597	22.1624	16.6100	15.2155	9.06538	2.78938
32.7458	30.0702	22.3687	17.0383	15.2960	9.08820	2.82976
32.7476	30.2688	22.5844	17.3746	15.4589	9.16338	2.83004
33.5753	30.4464	22.6480	17.9045	15.6564	9.25001	2.83866
33.9538	30.4902	22.6485	18.7881	15.7630	9.63293	2.87639
34.8950	31.0373	22.7139	19.3483	16.1859	9.69994	2.88687
36.1732	31.0507	23.0568	19.4518	16.3148	9.85730	2.89257
36.5595	31.2317	23.1565	19.4639	16.3396	9.86264	2.93173
36.6180	31.2418	23.3064	19.8900	17.0132	9.88294	2.93708
36.6865	31.2592	23.3518	20.1052	17.1270	9.98805	2.95099
36.9840	31.3931	23.4517	20.2601	17.8338	10.0244	2.96542
37.0481	31.4759	23.6615	20.3247	17.8524	10.0666	2.98098
37.0497	31.5123	23.6666	20.4498	18.1814	10.5096	3.06000
37.0918	32.0073	23.6930	20.7130	18.3851	10.6109	3.15139
37.4179	32.0210	23.9639	20.9164	18.4126	10.8614	3.28627
37.4329	32.2166	24.0208	20.9760	18.7779	11.1122	3.44215
37.7896	32.3910	24.3359	21.0985	18.8106	11.1916	3.45070
37.8363	32.4891	24.3579	21.3548	18.8602	11.2233	3.47092

Table A.9 (cont.)

38.0074	32.5067	24.4332	21.4924	19.4175	11.2996	3.48775
38.0901	32.5451	24.4953	21.6134	19.4743	11.3450	3.50252
38.1714	32.8819	24.6219	21.6796	19.4873	11.3473	3.50467
38.6683	32.9495	24.7630	21.7167	19.6706	11.3782	3.51975
38.7762	33.1883	24.8661	22.2350	19.7119	11.5394	3.58674
38.8846	33.3320	25.6433	22.3100	19.7931	11.5493	3.64870
39.0857	33.5571	25.6829	22.6690	20.0722	11.9294	3.71377
39.2014	33.6678	25.8288	22.7061	20.2132	12.1074	3.73607
39.7709	33.7213	26.0420	22.7291	21.3054	12.1401	3.74837
39.8708	34.2809	26.2160	22.8386	21.7946	12.2634	3.77347
39.8954	34.3155	26.2686	23.0784	21.8317	12.4554	3.79541
39.9835	34.6779	26.3431	23.3177	22.0827	12.5398	3.80658
40.2677	34.9066	26.4015	23.3449	22.2309	12.5846	3.96530
40.3796	34.9167	26.4074	23.4182	22.3942	12.6538	4.00805
41.3988	35.0517	26.5586	23.5775	22.6197	13.0230	4.19750
41.4772	35.0632	27.0839	23.7904	22.6476	13.2316	4.24142
41.5623	35.2705	27.2211	23.8470	23.2057	13.7922	4.30075
42.4664	35.3492	27.4263	23.9635	23.2384	13.8883	4.32007
42.5413	35.5804	27.5364	24.1406	23.2735	14.0589	4.33249
42.6192	35.8783	27.7433	24.7194	23.6096	14.2245	4.37861
42.6877	36.0168	27.8276	24.7736	23.7272	14.2481	4.42483
42.7072	36.1296	27.8531	24.9656	23.8526	14.3848	4.43445
42.9941	36.2919	27.8566	25.1626	23.9978	14.5426	4.48667
43.0316	36.7589	28.3814	25.4889	24.1084	14.5573	4.49213
43.1138	36.9352	28.4138	26.0375	24.2895	14.6003	4.82547
43.4831	37.0884	28.5123	26.0704	24.5929	14.9051	4.92107
43.6227	37.1409	28.9432	26.2964	24.7471	14.9544	5.01552
43.6761	37.1616	29.0950	26.3779	24.7651	15.5201	5.11605
43.9087	37.4394	29.4598	26.7462	25.0180	15.8073	5.18368
44.9779	37.7295	29.9847	26.9149	25.0551	15.8317	5.23983
45.3210	37.9197	29.9980	26.9598	25.2958	15.9602	5.29267
45.5224	38.0267	31.2111	27.3133	25.3540	15.9617	5.34297
45.5627	38.0754	31.3072	27.3433	25.5676	16.6244	5.34922
45.7633	38.0993	31.3566	27.6222	25.9095	16.7775	5.38703
45.9881	39.0376	31.3924	27.6661	25.9628	16.8364	5.42884
46.2682	39.6177	31.4672	27.7765	26.0966	17.0597	5.47761
46.3505	39.9239	31.5495	27.8355	26.2296	17.1345	5.51593
46.5112	40.0576	31.6005	28.1002	26.2651	17.2454	5.55947
47.0126	40.1980	31.6281	28.5070	26.3467	17.4345	5.61138
47.2192	40.3695	31.7329	28.9433	26.4278	18.0312	5.70738
47.4915	40.6116	32.0567	29.0212	26.6240	18.6502	5.72762
47.6575	41.2456	32.0880	29.1432	26.8505	18.9409	5.79326
47.7903	41.7210	32.4772	29.1799	26.8567	18.9767	5.81551
48.3575	41.7944	32.9687	29.3604	26.9123	19.1502	5.88925
48.7303	41.8048	33.0245	29.4495	26.9781	19.3530	5.92795
48.8565	41.8415	33.1442	29.4758	27.3763	19.4091	5.94040
48.9959	41.8615	33.2932	29.6343	27.3951	19.6307	6.05564
49.3783	41.8945	33.5672	29.6466	27.8578	19.7604	6.11916

Table A.9 (cont.)

49.7600	42.0627	33.7580	29.6522	28.1353	19.9352	6.55295
49.9521	42.1004	33.9563	29.7003	28.1446	19.9367	6.76777
50.0500	42.4171	34.0229	29.9318	28.6814	20.1245	7.02880
50.1951	42.6543	34.0697	30.0803	28.7096	20.1345	7.03739
50.2015	42.7259	34.3201	30.0948	29.5284	20.2852	7.04122
50.4818	42.7461	34.5204	30.4453	29.6680	20.4439	7.08427
50.9394	42.9416	34.5786	30.4853	29.7219	20.5065	7.28451
50.9808	43.2365	34.5819	30.8787	29.9432	20.5113	7.36935
51.0678	43.3166	34.9247	31.0430	31.0583	20.5714	7.37193
51.1238	43.5136	35.0997	31.4176	31.1293	20.7773	7.38807
51.1287	43.8060	35.2114	31.5005	31.2175	20.8233	7.39831
51.2752	44.0709	35.7364	31.5472	31.2494	20.9762	7.42322
51.4651	44.1619	35.8542	31.7667	31.3905	21.1819	7.50202
51.4958	44.4873	35.8545	31.7722	31.4023	21.1854	7.50701
51.6322	45.1525	36.0504	31.9539	31.4310	21.2933	7.67471
51.6910	45.2445	36.1016	31.9801	31.4512	21.4343	7.84899
52.1373	45.3204	36.1176	32.0314	31.5219	21.5231	7.99462
52.2630	45.6087	36.1405	32.3530	31.5445	21.8857	8.14083
52.4906	45.7078	36.2476	32.3834	31.6555	21.8971	8.27211
52.7858	45.7616	36.4550	32.4799	32.0773	22.1322	8.34287
52.8660	45.7700	36.5441	32.5882	32.5005	22.1480	8.40547
53.0635	45.9324	36.6901	32.7640	32.6703	22.5471	8.69030
53.7449	46.2495	36.7829	32.7880	33.1188	23.3712	8.71362
53.7935	46.2655	36.8615	32.8510	33.4488	23.6283	8.84133
53.9166	46.6744	37.1274	33.0121	33.8569	23.7329	8.96382
54.0197	46.9439	37.1560	33.0365	34.1474	23.9954	8.97643
54.0582	47.0015	37.4153	33.4250	34.3136	24.1342	9.02249
54.1240	47.2320	37.6346	33.5306	34.4537	24.2931	9.21168
54.2278	47.4570	38.0499	33.5859	34.5056	24.6151	9.29181
54.4909	47.7540	38.1544	33.6152	34.8377	24.9948	9.31940
54.7154	47.8086	38.4705	33.7944	34.8412	25.1358	9.34121
54.8134	47.8559	38.5024	34.2606	34.8993	25.2216	9.43300
54.8692	48.2588	39.1536	34.4688	35.2628	25.2611	9.43596
55.0530	48.2917	39.1907	34.5175	35.4945	25.6762	9.64557
55.4726	48.5301	39.2944	34.7112	35.5211	25.6966	9.74861
55.6380	48.7911	39.3474	34.8546	36.6653	25.7283	9.80327
55.7472	49.1832	39.4172	35.0785	36.6809	25.7496	9.88027
55.7956	49.2179	39.6354	35.2983	36.8387	25.7645	9.97572
56.3368	49.2777	39.9349	35.3947	37.1448	25.8052	10.0537
56.5637	49.5789	40.3059	35.6425	37.1778	25.8059	10.0826
56.5655	49.5844	40.4819	35.8944	37.2180	25.9854	10.1594
56.5831	50.1653	40.5327	36.0780	37.4736	26.3304	10.1701
56.6020	50.1777	40.5432	36.0805	37.5739	26.3476	10.1907
56.9362	50.2823	40.7099	36.0913	37.9673	26.3541	10.3024
57.0738	50.8877	40.8083	36.2927	38.1641	26.4322	10.5322
57.2749	51.1408	40.8307	36.3519	38.7954	26.4413	10.7269
57.5532	51.1820	40.8349	36.4849	39.0139	26.5867	10.9234
57.8516	51.2541	40.9508	36.6350	39.3222	26.7956	10.9501

Table A.9 (cont.)

58.0255	51.3321	41.0075	36.6581	39.3929	26.9448	11.0856
58.2236	51.6024	41.2045	36.8672	39.4158	27.0423	11.3196
58.3215	52.2566	41.2763	37.0163	39.9485	27.2056	11.3484
58.5824	52.3024	41.4808	37.0164	40.0235	27.5116	11.5067
59.0512	52.7866	41.5625	37.2510	40.4072	27.5935	11.7702
59.3541	52.7876	41.8255	37.9702	40.4898	27.8890	12.0572
59.5360	52.9559	41.8338	38.0272	40.5298	28.5532	12.1529
59.7306	52.9935	41.8399	38.0321	40.6386	28.5770	12.2765
59.8150	53.0707	42.0951	38.1751	41.0277	28.9734	12.2895
59.9738	53.0996	42.2938	38.4508	41.3491	29.1224	12.4436
60.1154	53.4178	43.0171	38.5498	41.5062	29.2077	12.5900
60.4733	54.5641	43.8801	39.1141	41.6187	29.3123	12.7068
60.8460	54.8820	44.1998	39.4180	41.7080	29.4822	12.9427
61.0028	54.9549	44.2870	39.5576	41.8918	29.5212	13.1544
61.0755	55.0334	44.3803	39.7293	41.9630	30.0154	13.3331
61.3336	55.1615	44.4428	39.9518	42.1379	30.2737	13.3413
61.3900	55.1670	45.2717	40.4270	42.1721	30.4504	13.7857
61.5923	55.2236	45.5883	40.6364	42.3364	30.4577	13.7881
61.6546	55.4797	45.8573	40.6484	42.3442	30.8363	13.8333
61.8674	55.5190	45.8635	40.6817	42.5094	31.4054	13.9936
61.8785	55.5641	45.9611	40.7775	42.5651	32.5476	14.3072
61.9073	55.6457	46.1760	40.8468	43.3316	32.6798	14.3582
61.9658	55.7468	46.2831	41.2560	43.3451	32.7531	14.5752
62.0057	55.7942	46.3112	41.8369	43.5799	32.8624	14.6129
62.4850	55.8356	46.3356	42.2079	43.9545	32.9291	14.6712
62.5925	55.8656	46.4190	42.7031	44.2642	33.5860	14.6841
62.8609	56.5266	46.4794	42.8201	44.3382	34.0036	14.9014
62.8916	56.5308	46.7071	43.0181	44.3824	34.0523	14.9128
63.1485	56.5687	46.7576	43.3398	44.4521	34.1903	15.0594
63.9912	56.6328	46.7651	43.4156	44.7017	34.4551	15.1188
64.5560	56.7211	46.8553	43.6742	44.7368	34.4661	15.3727
64.7010	56.7461	47.3622	43.7415	44.8445	34.6014	15.6355
64.8655	56.7968	47.6107	44.0684	45.1357	35.3167	15.6815
65.0034	57.3320	47.6826	44.1982	45.1536	35.5924	15.6981
65.6617	57.3551	48.3109	44.2841	45.6241	36.7391	16.1895
65.7021	57.5854	48.5296	44.3606	45.8081	36.9855	16.5355
65.7021	57.7525	48.7094	44.3735	45.9376	37.0805	16.6859
66.2952	59.1184	48.8250	44.4299	46.4048	37.0870	16.8008
66.5304	59.1955	48.9147	45.2948	47.0855	37.2236	16.8269
66.5309	59.2160	49.1616	45.4236	47.3383	37.6895	16.9414
66.7773	59.6014	49.9207	45.4480	47.7164	37.7654	17.0577
66.8183	60.1565	49.9565	45.5689	48.2522	38.0491	17.0973
66.9136	60.4918	50.0440	45.6170	48.4150	38.1754	17.3361
67.2066	60.9619	50.2186	46.1816	48.4256	38.3866	17.4057
67.7311	61.0211	50.4272	47.4518	48.8496	38.8390	17.4591
67.7843	61.4439	50.5309	47.5600	49.0347	39.0534	17.7778
67.8073	61.9906	51.0639	48.1668	49.2272	39.1612	18.7536
68.5261	62.0707	51.1315	48.6762	49.3316	39.2337	18.9134

Table A.9 (cont.)

68.5753	62.4578	51.2814	48.7641	49.5938	39.2666	18.9725
69.3540	62.6622	51.3861	48.9515	49.7886	39.2936	19.0767
69.7347	62.6653	51.3930	49.4909	49.9814	39.3557	19.2698
70.1019	62.9419	51.4606	49.5461	50.1450	39.6241	19.3560
70.3723	63.3545	51.4825	49.6371	50.3707	39.9607	19.9518
70.4142	63.9519	51.6661	49.7876	50.4514	40.7793	19.9722
71.8927	64.2040	51.9636	49.9411	50.9096	40.9883	20.1121
71.9317	64.2687	52.3269	50.0404	51.1070	41.5760	20.3552
72.2292	64.4566	52.4605	50.7054	51.3328	41.5849	20.7714
72.7077	64.8952	52.6997	50.8937	51.7779	41.6694	21.0058
73.2234	64.9622	52.8512	51.0957	52.2693	41.7234	21.9807
73.4491	65.1983	53.1988	51.1565	52.2847	42.3231	22.0767
73.6090	65.2128	53.2675	51.5730	52.5461	42.3718	22.3904
73.6830	65.5334	53.4861	51.9145	52.6362	42.7521	22.8992
73.7318	65.6481	53.7140	52.2462	52.7151	42.9056	23.0613
73.8894	66.4572	53.8273	52.7773	53.1008	43.0460	23.5965
73.9627	66.5596	54.4825	53.0758	53.4426	43.4368	23.9920
74.7190	67.0206	54.6954	53.6085	53.5443	43.6535	24.2225
75.7939	67.3117	54.8202	53.8413	53.8246	43.7241	24.8080
76.1410	67.8795	55.6392	53.9455	54.8670	43.9162	25.2756
76.9511	68.4192	55.6727	54.3477	55.1002	44.8783	25.4490
77.1603	69.0251	55.6800	54.8418	55.3162	45.2612	25.6696
77.6609	69.1856	55.7728	55.1589	56.0073	45.8009	25.9426
77.7020	69.5556	55.8806	55.4111	56.1623	46.2667	26.7344
78.2813	69.8512	55.8844	55.6483	56.8496	46.6093	27.2109
78.8585	70.0916	55.8999	56.1966	57.0949	47.0311	27.7002
79.1999	70.3770	56.0954	56.4948	57.1500	47.2793	28.0039
79.2170	70.8638	56.3610	57.0145	57.7653	47.9936	28.2598
79.2236	71.0921	57.5451	57.2820	58.3490	48.0843	28.3408
79.5874	71.1922	57.6248	57.2867	58.5968	48.2191	28.5545
79.9081	71.9249	57.7186	57.3572	58.7672	49.3524	28.6497
80.7570	73.1418	57.8671	57.8761	59.0504	50.2134	28.6924
80.9371	73.3117	58.8418	58.8086	59.2898	50.3625	29.2744
81.4611	73.8878	58.8481	59.0487	59.7813	50.9823	29.8901
82.4733	73.9528	59.3316	59.1463	59.9940	51.3339	30.1437
83.0166	75.3788	59.4371	59.2748	60.3607	51.8060	30.3062
83.2127	75.4605	59.4712	59.5441	60.4682	52.1857	30.6542
83.3079	75.4614	59.6114	59.7104	60.4845	52.6056	31.4537
83.5289	75.7871	60.2650	60.0081	60.8398	53.2590	31.5048
83.5681	76.1293	60.4709	60.0755	60.9320	53.3918	32.0212
84.4412	76.4186	60.6144	60.8206	60.9801	53.5548	32.4975
84.8584	77.4247	60.9986	61.1841	61.0861	55.3021	32.7388
85.1961	78.1238	62.4307	61.3963	61.2292	56.1149	32.7436
85.6690	79.6611	62.4503	62.4406	61.2435	57.9929	33.0213
85.9226	79.7674	63.5956	63.3578	61.4191	58.1528	33.2449
86.0946	79.9178	63.9928	63.5085	62.6262	58.6522	33.3605
86.3058	80.6922	64.6850	63.5725	65.7769	60.2884	33.4824
86.6068	82.2696	65.2867	65.1058	66.8333	61.7895	33.6537

Table A.9 (cont.)

86.6394	82.4904	65.7883	66.4859	67.5708	62.1971	33.9218
86.9955	82.6356	66.0979	66.7454	68.0959	62.3692	35.1177
87.1887	82.8691	67.2224	67.9959	68.3263	62.5107	39.2926
87.7490	82.9778	67.6439	68.2722	69.3842	62.8671	39.6555
88.4195	83.2235	67.7148	68.3186	72.5180	63.8531	39.8458
88.4476	83.3625	69.5364	70.1384	73.0610	63.9951	41.7221
88.5693	83.8520	71.1566	74.1158	73.1239	64.3122	42.7230
88.7060	84.0864	73.2490	75.9663	74.2562	65.2271	45.6129
88.7193	84.7074	74.1329	76.8967	74.7358	65.5544	51.2236
88.9589	84.9534	75.3101	77.4399	76.6413	65.6090	51.4708
89.2089	85.2147	79.1332	78.3958	77.9924	67.6042	55.7047
90.1395	85.5312	81.9663	78.4279	80.8780	68.7560	56.8447
90.1971	86.1444	84.8562	83.5083	81.0300	74.3100	65.2504
90.3992	88.6616	85.6530	86.7360	81.5380	75.5530	65.6011
91.6730	93.6343	85.8885	88.5826	85.7251	82.1206	74.8182
93.4791	95.2028	91.6959	93.3077	87.3148	90.1364	82.5973

Table A.10 Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with iodoacetic acid without and with 1, 2, 3, 4, 5 and 24 hour liquid-holding recovery.

LH_0h	LH_1h	LH_2h	LH_3h	LH_4h	LH_5h	LH_24h
2.22366	2.26277	0.191662	0.495869	0	0.294315	0
5.30440	2.40745	0.261931	0.677269	0.501511	0.358234	0
8.24983	3.74413	0.270235	0.687445	0.519606	0.362456	0
8.84009	3.79435	0.390688	0.736305	0.519933	0.489646	0
9.45031	4.88617	0.610922	0.744554	0.532629	0.503114	0
9.80738	4.89493	0.688975	0.764794	0.614943	0.518148	0
10.4836	5.13540	0.757676	0.782869	0.672697	0.573825	0
12.4220	5.26063	1.04870	0.809370	0.885445	0.576568	0.133527
13.1135	7.05863	1.08254	0.847884	0.946738	0.600313	0.228061
13.6057	7.23076	1.33898	0.882508	0.965346	0.602311	0.256325
13.6836	8.69497	1.34263	1.00805	0.992736	0.712896	0.261468
14.0771	8.88937	1.36292	1.06295	1.08409	0.733655	0.279522
14.5453	8.96562	1.44828	1.08123	1.16393	0.743638	0.333932
15.4418	9.36911	1.47295	1.12728	1.25782	0.819561	0.362280
15.7567	9.39547	1.61420	1.16583	1.35994	0.901277	0.403659
15.9679	9.54214	1.68235	1.27042	1.42985	0.926589	0.481601
16.0546	9.81670	1.69153	1.27566	1.45723	0.948348	0.485547
16.2403	9.82092	1.77528	1.33017	1.46009	0.961940	0.492235
16.3143	10.0180	1.87158	1.33155	1.56322	0.964763	0.536527
16.5563	11.2886	2.25932	1.38130	1.60183	1.01598	0.576431
17.1509	12.0991	2.35477	1.39820	1.63412	1.11308	0.581497
18.0973	12.1191	2.49750	1.45127	1.77459	1.17245	0.589785
18.6227	12.1509	2.61851	1.45653	1.80836	1.19688	0.621510
21.8385	12.1738	2.62798	1.49364	1.82971	1.23071	0.621515
22.2700	12.2983	2.67527	1.56335	1.88358	1.24152	0.630447
22.3949	13.0304	2.85674	1.56722	1.88796	1.26189	0.635906
22.4733	15.5188	2.98714	1.65384	1.95895	1.28300	0.685816
23.2834	15.5688	3.00601	1.71533	1.98464	1.34272	0.764381
23.3655	15.7862	3.06191	1.79170	2.00412	1.39081	0.787839
23.4819	15.9074	3.36803	1.83146	2.14423	1.39221	0.793161
24.1817	16.0034	3.38289	1.84602	2.25420	1.39928	0.874143
24.1958	16.0064	3.41406	1.85174	2.33398	1.41181	0.885594
24.3718	16.2421	3.48511	1.95125	2.36604	1.41189	0.908379
24.9594	16.9602	3.65882	1.97979	2.38091	1.45664	0.966828
25.2742	17.0076	3.69214	2.01081	2.56830	1.46445	0.985104
25.7348	18.0890	3.82058	2.01246	2.60606	1.50234	1.03289
25.9335	18.2445	3.90502	2.02778	2.76390	1.54768	1.08296
26.0804	18.3219	4.19202	2.10397	2.78009	1.71480	1.13192
26.3425	18.3234	4.25757	2.19112	2.86798	1.72883	1.19086
26.4546	18.6216	4.31895	2.30414	2.91371	1.74949	1.20109
26.7943	18.6625	4.33826	2.32739	3.02680	1.75075	1.20880
26.8677	19.2516	4.36582	2.33359	3.37306	1.77403	1.45025
27.8646	19.8730	4.37891	2.38529	3.39586	1.87982	1.50156
28.1732	20.2874	4.39181	2.49231	3.46701	1.95945	1.58241
28.3549	20.5267	4.52444	2.61448	3.51537	2.01653	1.63030

Table A.10 (cont.)

28.6677	21.1950	4.53555	2.69089	3.55230	2.04196	1.75011
28.7559	21.8772	4.63244	2.69688	3.62260	2.05107	1.79595
28.8729	21.9754	4.69212	2.73625	3.74788	2.20814	1.83774
30.4823	22.1218	4.69271	2.76675	3.97260	2.27746	1.94480
30.7282	22.1439	4.84426	2.78093	4.15777	2.34846	2.17728
31.0324	23.0875	5.04250	2.78176	4.21699	2.36129	2.24634
31.1610	23.7659	5.35286	2.95738	4.33617	2.43982	2.25898
31.3572	23.9770	5.67237	2.98074	4.35641	2.46731	2.26563
31.4106	24.0045	5.71534	2.98408	4.53913	2.49335	2.35011
31.7611	24.1165	5.73507	3.08498	4.88923	2.50019	2.36254
31.8499	24.2412	5.92996	3.28843	4.89806	2.56097	2.43877
32.3778	24.2932	6.12192	3.29418	4.91451	2.60234	2.44152
32.3937	24.3450	6.54870	3.36461	5.11232	2.68148	2.44360
33.8527	25.2099	6.94649	3.45318	5.36065	2.72855	2.61709
33.8643	25.5997	7.18128	3.48553	5.39335	2.76314	2.72706
34.2656	25.7288	7.42753	3.60953	5.44585	2.83373	2.79663
34.8311	26.2373	7.57491	3.63507	5.46974	2.83429	2.92629
34.9261	26.4843	7.64612	3.65816	5.53013	3.00264	2.99729
35.0854	26.7701	7.98822	3.71106	5.54137	3.06344	3.03024
35.6064	26.8970	8.01106	3.78840	5.69404	3.29277	3.11075
35.6212	26.9500	8.09944	3.79520	5.75746	3.31436	3.13070
36.0997	27.3577	8.22125	3.80353	5.83001	3.61870	3.24339
36.5583	27.8554	8.28117	3.94298	6.31277	3.62521	3.26110
36.7910	28.1782	8.40055	4.01680	6.42983	3.62722	3.35492
36.8172	28.4018	8.42914	4.05545	6.45713	3.77870	3.54287
37.2319	28.9597	8.45348	4.08676	6.60595	3.87009	3.75743
37.2680	29.1164	8.47059	4.15903	7.03956	3.93339	3.91350
37.3269	29.1449	8.65895	4.20612	7.06331	4.07581	3.93388
38.1631	29.1997	8.68041	4.30869	7.12873	4.19748	4.00938
38.4654	29.6072	8.75122	4.43395	7.20060	4.19960	4.03959
39.0385	29.8486	8.94127	4.54216	7.20317	4.23518	4.06907
40.0270	30.0662	9.06070	4.58513	7.24176	4.24486	4.34938
40.0428	30.2713	9.38983	4.60144	7.27155	4.25317	4.45390
41.1870	30.3491	9.39381	4.67944	7.53188	4.26286	4.60309
41.2221	30.5080	9.53549	4.83108	7.79957	4.26658	4.70645
42.0323	30.5425	9.68454	4.83715	7.82491	4.31949	4.86115
42.1312	30.9212	10.2106	4.89323	8.16614	4.33107	4.92435
42.2395	31.0017	10.3256	4.92849	8.17564	4.39326	4.95841
42.9873	31.0202	10.5050	4.97815	8.20174	4.51031	5.10819
43.0961	31.0814	10.6906	4.98021	8.38555	4.53895	5.25226
43.3106	31.0972	10.7181	5.09863	8.44147	4.62430	5.49695
43.9618	31.7754	10.8296	5.29670	8.44794	4.82492	5.61583
44.4299	32.8873	11.2031	5.32650	8.48874	5.08124	5.94650
44.6279	32.9689	11.3700	5.44335	8.48979	5.15353	6.00391
45.0516	33.0010	11.5154	5.45129	8.66336	5.16505	6.05233
45.0821	33.0676	11.5403	6.08192	8.75502	5.35986	6.24773
45.0856	33.1392	11.7826	6.25059	8.78797	5.47048	6.38891
45.1341	33.1495	11.9123	6.31737	8.80531	5.50540	6.74108

Table A.10 (cont.)

45.1878	33.5063	11.9200	6.52274	9.07941	5.60133	6.76008
45.5111	33.6396	12.0308	6.74377	9.18809	5.63165	6.77059
45.8574	33.6575	12.0739	6.77978	9.21217	5.63656	6.87237
45.9766	33.9897	12.2775	7.13467	9.36759	5.78487	7.06629
46.2256	34.3478	12.6380	7.19471	9.41906	5.99335	7.15848
47.6191	34.6175	12.7439	7.20541	9.54469	5.99729	7.25515
47.6949	34.8688	13.0246	7.48919	9.59345	6.04834	7.33618
47.7699	34.9163	13.0293	7.58127	9.61647	6.16097	7.52769
47.9861	35.0302	13.1085	7.73621	9.69869	6.19511	7.60133
48.3197	35.3997	13.4776	7.83210	9.89738	6.27348	7.92392
48.6630	35.4310	13.7887	7.95255	10.0426	6.27841	8.10108
48.7973	35.6130	14.4750	8.06993	10.1325	6.44526	8.18255
49.2755	35.6884	14.6212	8.12250	10.2925	6.67614	8.25717
49.8778	35.7550	14.6580	8.25609	10.3107	6.73133	8.32422
50.8032	35.9430	14.6591	8.27785	10.3716	6.78031	8.36446
51.0269	35.9731	14.7746	8.36591	10.5662	7.05665	8.42749
51.3365	35.9999	14.9545	8.53873	10.6124	7.20839	8.79240
52.3686	36.2915	15.0150	9.01559	10.6328	7.37112	8.83174
52.7389	36.3844	15.4134	9.51640	10.9288	7.57697	9.00951
52.8835	36.5579	15.4726	9.53841	10.9451	7.78274	9.12581
53.4387	36.6209	15.5063	9.56033	10.9629	7.79228	9.24972
53.7082	37.0802	15.5489	9.61734	11.0921	7.84977	9.31150
53.9663	37.2093	15.6403	9.71153	11.1656	7.89077	10.1502
54.0031	37.5283	15.7705	9.94892	11.2058	7.93650	10.1904
54.0598	38.2833	16.2890	10.0820	11.3651	8.19500	10.4880
54.1982	38.3062	16.5048	10.0978	11.9837	8.26329	11.3820
54.2043	38.5923	16.5905	10.1752	12.1779	8.26407	11.3881
54.3797	38.7478	17.0791	10.2120	12.3556	8.31208	11.4539
54.5246	38.7777	17.1148	10.3632	12.3709	8.48978	11.8324
54.5654	38.8520	17.1826	10.3976	12.5522	8.65767	11.9282
54.8257	38.9167	17.3306	10.4560	12.5663	8.70505	12.0869
55.0921	39.4589	17.5653	10.6978	12.8231	8.93084	12.1730
55.3997	40.3686	17.7827	10.7993	12.8504	8.96973	12.3810
55.7869	40.5194	17.8478	10.8575	12.9548	9.05218	12.6446
55.8580	40.6295	17.9647	11.0472	13.3445	9.14169	13.2540
56.1786	40.6872	18.0285	11.5288	13.5310	9.19518	14.0117
56.3113	40.7915	18.1994	11.7937	13.7779	9.28882	14.0324
56.4344	40.9117	18.3722	11.9264	14.0270	9.38376	15.1126
56.8593	41.0952	18.4127	12.0070	14.1155	9.72480	15.6228
56.9508	41.1785	18.8956	12.1354	14.1185	10.0724	16.2674
57.2437	41.9929	19.7957	12.1644	14.3375	10.2169	18.5396
57.6181	42.2130	20.0194	12.3407	14.4198	10.2938	19.0951
57.7113	42.3657	20.1842	12.6011	14.4692	10.5188	19.4643
57.8692	42.4771	20.7020	12.9851	14.5522	10.5733	19.4855
57.8796	43.1974	21.1696	13.0878	14.8344	10.6512	20.2319
57.9044	43.8366	21.2595	13.1272	14.9363	10.6990	20.7542
57.9085	44.4447	21.5157	13.3242	15.1795	10.7475	21.5910
57.9753	44.5647	21.5325	13.3969	15.2171	10.9302	21.8347

Table A.10 (cont.)

58.1144	44.6123	21.5481	13.6723	15.4249	10.9406	24.3817
58.5550	44.6225	21.8425	14.0182	15.4736	11.2157	27.4797
58.6982	45.3260	21.8743	14.0388	15.6340	11.2229	27.9591
58.8343	45.9841	22.1027	14.1654	15.7045	11.2337	30.2278
59.6550	46.0397	22.4232	14.1873	15.7155	11.3612	35.3916
60.5560	46.2245	22.7151	14.2040	16.1462	11.6631	39.4776
61.6742	46.4054	23.4321	14.2801	16.1575	12.0045	42.4262
61.7313	46.5746	23.5357	14.3680	16.1946	12.0446	50.7432
62.1088	47.1524	23.9130	14.6113	16.5830	12.2114	71.4806
62.1263	47.8480	24.0608	14.7741	16.6449	12.2695	
62.3355	48.2593	24.2904	14.8942	16.7304	12.2738	
62.4591	49.2181	24.3109	14.9211	16.9607	12.3028	
62.4684	49.6425	24.4120	15.1454	18.0632	12.6226	
62.9277	49.7757	24.5329	15.2544	18.3102	12.6710	
62.9384	49.9801	24.9660	15.8155	18.5397	12.9277	
63.0238	50.0069	25.2274	16.0025	19.7977	12.9645	
63.0255	50.0570	25.3503	16.0493	19.8317	13.1518	
63.5592	50.0617	25.4203	16.3148	20.6160	13.5998	
63.7292	50.1941	25.5667	16.5383	21.0144	13.8931	
63.7503	50.2960	25.6538	16.6414	21.3090	14.6890	
63.8291	50.7695	25.7679	16.9034	21.5066	15.0809	
63.9805	50.7942	25.8106	16.9365	21.8911	15.2060	
64.1590	51.3394	26.3319	17.0591	21.9165	15.3770	
64.3231	51.6391	26.5977	17.3028	22.1731	15.6736	
64.3378	51.6889	26.6735	17.4617	22.5452	15.9949	
64.3467	53.2729	26.7095	17.5773	23.4207	16.5505	
64.4020	53.6079	26.8437	17.7073	23.8012	17.1427	
64.5918	53.6471	27.0481	18.0084	23.9633	17.5738	
64.6118	54.0124	27.0494	18.3593	24.1133	17.8371	
64.6976	54.0933	27.9309	19.0061	24.3795	17.8812	
64.7371	54.6168	28.3039	19.5767	24.7636	18.4507	
64.9955	54.6573	28.9528	19.7679	25.0736	18.9634	
65.0337	54.7360	29.5485	19.8789	25.1317	19.8031	
65.0602	54.8026	29.5810	20.1791	25.1684	20.4408	
65.1231	55.1988	29.6518	20.2790	25.1996	21.0489	
65.2140	55.2483	30.6926	20.4827	25.2497	21.0844	
65.3120	55.4508	31.0524	21.6162	25.5584	21.1801	
65.5518	55.7827	31.4328	21.7802	26.3794	21.2434	
65.6320	55.8689	32.2772	22.2766	26.5584	21.2857	
65.7636	56.0542	32.3260	22.7078	27.3556	21.3155	
65.7774	56.1296	32.5775	23.4588	27.6263	21.5734	
65.8174	56.1398	33.7345	23.6234	27.6636	22.1570	
65.8340	56.4757	33.9139	24.3865	27.7616	22.1594	
65.9150	56.8632	34.0260	24.6469	28.5628	22.3842	
66.3143	56.9968	34.7883	24.7310	28.6904	22.4094	
66.6854	57.1358	34.9244	24.9439	28.8977	22.6406	
66.9221	57.2315	35.3789	25.3361	29.5182	23.4184	
67.0853	57.4757	35.3844	25.6013	29.6093	23.9455	

Table A.10 (cont.)

67.4171	57.5958	36.3950	26.1077	30.1477	24.4355
68.0131	57.9643	37.0430	26.6048	30.9938	24.4393
68.1016	58.1159	37.4164	26.6059	31.0024	24.5621
68.2652	58.5143	37.9735	26.9356	31.0301	24.9081
68.4007	58.8007	38.2164	27.2518	31.4271	24.9543
68.6030	59.3306	39.2080	28.7344	31.8736	25.0240
68.6132	59.3758	39.4035	28.9095	31.9559	25.0822
69.4010	59.4657	39.9929	29.3894	32.3917	25.2448
69.4205	60.0386	40.4928	29.4222	32.9550	25.3344
69.6263	60.0969	40.8771	29.5364	33.0116	25.4404
69.8781	60.3800	41.0267	29.9164	33.0644	25.4991
69.9650	60.6541	41.3016	30.2188	33.6212	26.4372
70.0874	60.7439	42.1899	31.2710	34.1411	26.6523
70.2505	60.8490	42.2441	31.8965	34.2349	26.8793
70.4244	60.9868	42.3129	34.8671	34.3581	26.9194
70.4599	61.5635	42.7147	35.8793	34.4268	27.4073
70.6483	61.8760	43.1162	36.1658	34.7563	27.4271
70.7071	61.9303	43.2866	37.1526	34.8642	27.4492
70.7231	62.3470	43.7904	37.6977	34.9344	27.9573
70.7476	63.5093	43.8835	38.1764	35.0029	28.6965
71.0015	63.6471	44.3268	38.1768	35.1196	29.8750
71.4349	63.7578	44.6765	38.2550	35.1748	30.3530
71.4661	63.8262	44.6776	38.3448	35.5531	30.5576
71.8793	64.6103	44.7143	38.6741	35.7780	31.5217
72.0203	65.8693	45.3217	39.2970	35.8050	32.8219
72.1775	66.9638	46.3604	39.7809	37.2512	33.0650
72.4639	67.0608	46.7027	39.8259	39.0717	34.4309
72.6216	67.7946	46.7978	40.0956	39.3716	34.6582
72.6238	67.9951	46.9447	40.2220	39.8367	34.7736
72.6334	68.1762	47.0083	40.4294	40.9133	35.1722
72.7792	68.5564	47.5504	40.6030	41.0550	35.4923
72.7956	69.5252	48.4033	40.8476	41.7600	37.1772
73.0187	69.6063	48.9351	42.5751	42.3474	37.9983
73.4030	69.7016	48.9779	43.3797	42.4843	38.0056
73.5999	69.7450	49.5010	45.0503	42.5524	39.0511
73.6530	70.8413	50.9597	45.5207	43.1610	39.0747
73.7855	71.3925	51.2579	46.5836	44.1992	39.1466
73.7861	72.3441	51.5829	48.5332	45.1845	39.6667
73.8170	73.1115	52.1891	49.2604	45.2411	39.7650
73.8638	73.4360	52.7037	49.6855	45.3765	41.3455
74.0034	73.8021	53.2836	49.9456	45.5622	41.5941
74.3542	73.8580	55.3207	51.3217	45.7247	41.9627
74.4482	73.8624	55.3611	52.1553	45.9350	42.1522
74.4553	74.4017	56.3469	52.7183	46.0441	42.1616
74.6182	75.6144	56.5020	54.2287	47.0904	42.8367
74.7221	75.9732	57.0286	54.4678	47.7622	45.6791
74.9172	76.2113	57.1103	55.8198	47.9738	45.7965
75.0722	76.8931	57.3358	56.0154	48.0259	45.9790

Table A.10 (cont.)

75.3959	77.1737	58.2410	56.6955	49.1139	46.8808
75.6692	77.4431	59.7715	57.4124	51.2145	48.4898
75.8094	78.3312	61.3750	58.8668	54.8246	49.8784
75.8877	79.1541	62.8625	59.1486	55.8622	51.9324
75.9284	79.5003	64.6122	63.7716	56.6409	52.2045
76.6778	80.7513	72.5494	64.8556	57.6698	58.3346
76.8100	81.3215	73.5204	67.4691	57.9968	58.7768
76.9920	82.0075	75.1110	69.3642	60.4418	59.6570
77.2256	82.8830	77.3333	71.3724	61.0264	60.2691
77.2810	83.4124	80.1169	72.9057	63.5908	63.6108
77.6559	83.6813	80.3934	78.8736	74.5732	66.9671
77.6579	89.2887	83.8889	84.9899	76.7490	79.2056
77.7864	90.5201	89.2179	89.0381	77.2850	83.5153
77.9528					
77.9661					
77.9967					
78.0530					
78.1601					
78.3463					
78.5871					
78.7394					
78.7905					
78.9358					
79.2068					
79.2687					
79.2719					
79.3565					
79.4951					
80.1991					
80.8129					
80.8307					
81.0456					
81.0890					
81.1930					
81.2041					
81.3072					
81.3390					
81.3423					
81.4803					
81.4925					
81.5652					
81.7789					
81.7852					
81.8519					
81.9088					
82.0255					
82.1559					
82.5918					

Table A.10 (cont.)

82.6019
82.6880
82.7190
82.8155
82.9375
83.0475
83.0555
83.2049
83.2602
83.6022
83.6405
83.6579
83.8015
84.1311
84.1581
84.2934
84.3495
84.7251
85.0776
85.1268
85.5163
85.5613
85.5719
85.6982
85.7564
85.8332
86.1233
86.2920
86.3442
86.3629
86.6348
87.0410
87.1347
87.4011
87.5138
87.5327
87.6047
87.7767
87.9164
88.6335
88.6469
89.0639
89.0804
89.4531
89.4896
89.6863
90.2221
90.2363

Table A.10 (cont.)

90.4960
90.5902
90.8106
90.9084
91.0229
91.1310
91.7309
91.7540
92.2705
93.0282
93.4731
93.6651
93.8386
94.4862
94.5506
95.0381
96.0386

Table A.11 Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from the CHO cell negative controls at 0, 5, and 24 hour liquid-holding recovery.

LH_0h	LH_5h	LH_24h
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0.1217
0	0	0.1422
0	0.1234	0.1619
0	0.1411	0.2085
0	0.1532	0.2234
0	0.1555	0.2274
0	0.1588	0.2288
0	0.1630	0.2308
0	0.1649	0.2350
0	0.1687	0.2366
0	0.1815	0.2471

Table A.11 (cont.)

0	0.1888	0.2567
0	0.1950	0.2578
0	0.2044	0.2681
0	0.2051	0.2722
0	0.2134	0.2762
0	0.2155	0.2785
0	0.2162	0.2880
0	0.2175	0.2996
0	0.2196	0.3036
0	0.2227	0.3220
0	0.2292	0.3235
0	0.2312	0.3251
0	0.2385	0.3318
0	0.2446	0.3369
0	0.2512	0.3376
0.0759	0.2518	0.3419
0.1167	0.2534	0.3440
0.1276	0.2563	0.3574
0.1398	0.2627	0.3590
0.1437	0.2668	0.3679
0.1451	0.2731	0.3696
0.1629	0.2755	0.3776
0.1816	0.2783	0.3844
0.1830	0.2789	0.4009
0.1841	0.2801	0.4035
0.1889	0.2814	0.4041
0.1912	0.2824	0.4057
0.1933	0.2865	0.4084
0.1966	0.2903	0.4089
0.1969	0.2935	0.4198
0.2068	0.2991	0.4237
0.2082	0.3024	0.4257
0.2084	0.3075	0.4418
0.2104	0.3098	0.4447
0.2141	0.3102	0.4482
0.2153	0.3114	0.4635
0.2174	0.3124	0.4747
0.2176	0.3137	0.4771
0.2187	0.3156	0.4819
0.2203	0.3178	0.4861
0.2213	0.3179	0.4883
0.2239	0.3181	0.4999
0.2243	0.3224	0.5115
0.2265	0.3302	0.5136
0.2265	0.3409	0.5206
0.2281	0.3429	0.5322
0.2285	0.3474	0.5344
0.2310	0.3481	0.5379

Table A.11 (cont.)

0.2338	0.3484	0.5571
0.2349	0.3487	0.5597
0.2350	0.3560	0.5665
0.2368	0.3619	0.5833
0.2371	0.3624	0.5861
0.2381	0.3651	0.5887
0.2416	0.3772	0.5944
0.2428	0.3821	0.5970
0.2442	0.3866	0.6019
0.2522	0.3902	0.6048
0.2549	0.3948	0.6256
0.2560	0.3962	0.6381
0.2570	0.4017	0.6417
0.2577	0.4033	0.6643
0.2584	0.4068	0.6644
0.2605	0.4104	0.6794
0.2630	0.4158	0.6962
0.2661	0.4184	0.6983
0.2674	0.4192	0.7188
0.2680	0.4206	0.7232
0.2758	0.4248	0.7274
0.2767	0.4295	0.7325
0.2787	0.4316	0.7382
0.2852	0.4461	0.7820
0.2876	0.4464	0.7910
0.2877	0.4470	0.7912
0.2889	0.4560	0.7920
0.2900	0.4576	0.8079
0.2920	0.4613	0.8145
0.2950	0.4671	0.8166
0.2970	0.4684	0.8401
0.2976	0.4710	0.8623
0.2996	0.4756	0.8665
0.3024	0.4935	0.8669
0.3051	0.4958	0.8736
0.3059	0.5047	0.8861
0.3085	0.5056	0.8934
0.3137	0.5163	0.8947
0.3158	0.5344	0.9118
0.3185	0.5351	0.9202
0.3196	0.5423	0.9290
0.3314	0.5430	0.9320
0.3337	0.5451	0.9394
0.3384	0.5459	0.9603
0.3428	0.5489	0.9609
0.3466	0.5492	0.9646
0.3514	0.5539	0.9728
0.3519	0.5539	0.9842

Table A.11 (cont.)

0.3521	0.5576	0.9870
0.3531	0.5604	1.0329
0.3543	0.5610	1.0362
0.3559	0.5639	1.0427
0.3603	0.5718	1.0480
0.3605	0.5724	1.0606
0.3630	0.5728	1.0801
0.3682	0.5730	1.0885
0.3706	0.5735	1.1046
0.3712	0.5766	1.1116
0.3724	0.5784	1.1351
0.3731	0.5933	1.1389
0.3766	0.5950	1.1437
0.3784	0.6020	1.1455
0.3810	0.6064	1.1522
0.3811	0.6096	1.1536
0.3823	0.6109	1.1553
0.3861	0.6147	1.1747
0.3889	0.6283	1.2237
0.3933	0.6326	1.2263
0.3978	0.6395	1.2532
0.4000	0.6402	1.2616
0.4051	0.6477	1.2809
0.4076	0.6572	1.2839
0.4102	0.6598	1.2926
0.4131	0.6777	1.3009
0.4227	0.6793	1.3073
0.4229	0.6852	1.3177
0.4264	0.6860	1.3217
0.4278	0.6880	1.3248
0.4298	0.6892	1.3265
0.4306	0.6951	1.3537
0.4356	0.6984	1.3616
0.4388	0.6994	1.3746
0.4424	0.7050	1.3908
0.4464	0.7059	1.3930
0.4469	0.7184	1.4248
0.4496	0.7253	1.4487
0.4528	0.7264	1.4774
0.4601	0.7327	1.4784
0.4602	0.7337	1.4912
0.4627	0.7499	1.4972
0.4639	0.7542	1.5054
0.4663	0.7545	1.5074
0.4705	0.7570	1.5771
0.4723	0.7608	1.5935
0.4723	0.7616	1.6836

Table A.11 (cont.)

0.4818	0.7636	1.6847
0.4821	0.7646	1.6911
0.4854	0.7667	1.6985
0.4873	0.7707	1.7188
0.4890	0.7726	1.7197
0.4892	0.7730	1.7294
0.4910	0.7730	1.8149
0.4921	0.7767	1.8310
0.4928	0.7786	1.8486
0.4956	0.7798	1.8528
0.5007	0.7929	1.8617
0.5082	0.8023	1.8816
0.5092	0.8089	1.9202
0.5188	0.8133	1.9284
0.5189	0.8178	1.9346
0.5209	0.8211	1.9531
0.5211	0.8214	1.9853
0.5226	0.8361	2.0222
0.5369	0.8389	2.0621
0.5442	0.8485	2.0746
0.5445	0.8499	2.0899
0.5499	0.8516	2.1464
0.5542	0.8587	2.1937
0.5590	0.8610	2.2242
0.5632	0.8613	2.2257
0.5668	0.8749	2.2458
0.5739	0.8796	2.2626
0.5762	0.8803	2.2685
0.5814	0.8859	2.3055
0.5819	0.8889	2.3224
0.5823	0.8953	2.3238
0.5837	0.9040	2.5038
0.5850	0.9097	2.5227
0.5906	0.9099	2.5400
0.5907	0.9125	2.5778
0.5992	0.9164	2.5939
0.6005	0.9164	2.5940
0.6050	0.9175	2.6814
0.6052	0.9250	2.6878
0.6073	0.9259	2.6900
0.6158	0.9412	2.7046
0.6179	0.9518	2.7512
0.6182	0.9525	2.7550
0.6192	0.9552	2.8100
0.6269	0.9599	2.8473
0.6358	0.9752	2.9419
0.6368	0.9753	2.9759
0.6369	0.9816	3.0335

Table A.11 (cont.)

0.6420	0.9846	3.0600
0.6485	0.9849	3.0884
0.6574	0.9857	3.1861
0.6581	0.9878	3.2045
0.6603	0.9923	3.3181
0.6669	0.9937	3.3237
0.6739	0.9939	3.3826
0.6750	0.9957	3.3988
0.6817	0.9999	3.4288
0.6849	1.0000	3.4782
0.6852	1.0003	3.4889
0.6859	1.0042	3.5575
0.6876	1.0076	3.5610
0.6972	1.0132	3.8412
0.6978	1.0204	3.9141
0.6995	1.0249	3.9582
0.7079	1.0588	4.0549
0.7117	1.0630	4.0916
0.7140	1.0653	4.2410
0.7266	1.0828	4.2957
0.7306	1.0855	4.3772
0.7309	1.0891	4.3866
0.7337	1.1088	4.4268
0.7353	1.1369	4.4478
0.7416	1.1399	4.5167
0.7508	1.1582	4.5537
0.7538	1.1597	4.5566
0.7603	1.1599	4.6008
0.7649	1.1741	4.7211
0.7659	1.1762	4.7805
0.7728	1.1807	4.7938
0.7748	1.1870	4.9725
0.7773	1.1895	4.9799
0.7916	1.1903	5.0223
0.7937	1.1932	5.0468
0.7958	1.1951	5.0695
0.7964	1.2059	5.1180
0.7974	1.2069	5.1364
0.8012	1.2077	5.1491
0.8042	1.2143	5.1679
0.8074	1.2241	5.2120
0.8099	1.2379	5.2561
0.8117	1.2652	5.2602
0.8139	1.2781	5.3141
0.8210	1.2831	5.3638
0.8218	1.3043	5.4277
0.8256	1.3065	5.6390
0.8278	1.3120	5.6610

Table A.11 (cont.)

0.8289	1.3170	5.6855
0.8298	1.3230	5.8042
0.8313	1.3253	5.8066
0.8392	1.3312	5.8317
0.8628	1.3553	5.8419
0.8644	1.3663	5.8792
0.8678	1.3942	5.9764
0.8821	1.3972	6.1644
0.8901	1.4309	6.1936
0.8956	1.4435	6.2322
0.8963	1.4932	6.3635
0.8970	1.4958	6.4953
0.9153	1.4982	6.6558
0.9153	1.5219	6.8086
0.9343	1.5493	6.8317
0.9356	1.5512	6.9399
0.9377	1.5522	6.9656
0.9395	1.5690	6.9955
0.9403	1.5696	6.9974
0.9449	1.5804	7.2889
0.9473	1.5809	7.3087
0.9482	1.5975	7.3132
0.9641	1.6142	7.3519
0.9688	1.6171	7.4154
0.9723	1.6183	7.6750
0.9760	1.6317	7.7818
0.9896	1.6382	7.8130
0.9950	1.6558	7.8188
0.9979	1.6582	7.8831
1.0016	1.6723	7.9175
1.0064	1.6764	7.9409
1.0101	1.6867	8.0829
1.0104	1.6909	8.1009
1.0143	1.6917	8.1268
1.0254	1.7244	8.2246
1.0384	1.7358	8.2503
1.0430	1.7363	8.2560
1.0439	1.7601	8.3565
1.0465	1.7607	8.3587
1.0524	1.7673	8.4154
1.0575	1.7743	8.5353
1.0661	1.7766	8.7370
1.0677	1.7846	8.8778
1.0767	1.8021	8.8805
1.0817	1.8131	8.9188
1.0929	1.8145	8.9195
1.0970	1.8223	8.9584
1.1039	1.8264	9.0177

Table A.11 (cont.)

1.1144	1.8331	9.1504
1.1145	1.8499	9.3068
1.1261	1.8644	9.3300
1.1297	1.8705	9.4082
1.1312	1.8864	9.4213
1.1416	1.9658	9.5363
1.1569	1.9704	9.5540
1.1570	1.9902	9.5743
1.1604	2.0033	9.7333
1.1650	2.0088	9.7344
1.1740	2.0393	9.7725
1.1790	2.0926	10.1513
1.1843	2.1010	10.2725
1.1934	2.1107	10.3189
1.1977	2.1308	10.3424
1.2254	2.1388	10.4523
1.2298	2.1827	10.5307
1.2304	2.1834	10.5669
1.2408	2.2124	10.6122
1.2594	2.2297	10.6348
1.2891	2.2595	10.7634
1.2904	2.2814	10.8987
1.2918	2.2967	10.9072
1.3152	2.3258	10.9560
1.3275	2.3446	10.9589
1.3285	2.3588	10.9702
1.3290	2.3734	11.1034
1.3348	2.4375	11.4359
1.3371	2.4377	11.5769
1.3468	2.4593	11.5821
1.3507	2.4634	11.8473
1.3569	2.4650	11.9577
1.3605	2.4702	11.9964
1.3635	2.4908	12.0229
1.3911	2.4993	12.2064
1.4154	2.5854	12.3419
1.4208	2.5900	12.4237
1.4269	2.6238	12.5325
1.4459	2.6679	12.6395
1.4502	2.6947	12.6866
1.4518	2.6965	12.7362
1.4628	2.7546	12.8717
1.4646	2.7564	12.9625
1.4761	2.7769	13.2463
1.4792	2.7825	13.4107
1.4811	2.7831	13.6657
1.4917	2.7994	13.8560
1.5002	2.8399	13.9620

Table A.11 (cont.)

1.5104	2.8852	14.6061
1.5293	2.9327	15.4134
1.5370	2.9609	15.4435
1.5723	2.9679	15.5653
1.5928	2.9756	15.8171
1.5976	3.0206	16.2705
1.6026	3.0919	16.3645
1.6068	3.1118	16.5015
1.6072	3.1988	16.5559
1.6127	3.2449	16.7784
1.6185	3.2750	16.8602
1.6243	3.2938	17.0111
1.6251	3.3127	17.4043
1.6559	3.3229	18.2429
1.6672	3.3391	18.5106
1.6694	3.3530	18.7334
1.6696	3.3586	19.6267
1.6843	3.3822	19.7726
1.6884	3.3953	21.0635
1.6937	3.4342	22.8917
1.7023	3.4367	24.2786
1.7285	3.4916	
1.7301	3.4926	
1.7413	3.5028	
1.7577	3.5045	
1.7619	3.5269	
1.7680	3.5415	
1.7700	3.5432	
1.7729	3.5628	
1.7920	3.5660	
1.7997	3.5922	
1.8019	3.6106	
1.8095	3.6407	
1.8170	3.6415	
1.8184	3.6526	
1.8405	3.6534	
1.8407	3.6780	
1.8543	3.6834	
1.8689	3.6945	
1.8750	3.7206	
1.8828	3.7572	
1.8883	3.7591	
1.8925	3.8304	
1.9325	3.8381	
1.9985	3.8658	
2.0739	3.8715	
2.0740	3.8852	
2.0899	3.9314	

Table A.11 (cont.)

2.0903	3.9586
2.1689	3.9626
2.1692	3.9700
2.1736	3.9887
2.1798	3.9909
2.1838	3.9918
2.1984	4.0107
2.2096	4.0696
2.2142	4.0878
2.2268	4.0911
2.2269	4.0957
2.2335	4.1034
2.2367	4.1190
2.2451	4.1221
2.2572	4.1398
2.2573	4.2022
2.2754	4.2294
2.2790	4.2453
2.2837	4.2574
2.2856	4.2652
2.2919	4.2881
2.3006	4.3079
2.3217	4.3241
2.3229	4.3516
2.3266	4.4065
2.3273	4.4854
2.3370	4.4976
2.3721	4.5764
2.3762	4.5966
2.3837	4.6023
2.4014	4.6027
2.4093	4.7260
2.4142	4.7324
2.4471	4.9016
2.4688	4.9435
2.4705	4.9616
2.4769	4.9771
2.4986	5.0054
2.5072	5.0397
2.5101	5.0458
2.5112	5.0740
2.5338	5.1064
2.5757	5.1074
2.6098	5.1294
2.6159	5.1814
2.6400	5.2274
2.6606	5.2783
2.6775	5.2862

Table A.11 (cont.)

2.6870	5.3027
2.6901	5.3051
2.7102	5.3241
2.7105	5.4925
2.7171	5.5218
2.7323	5.5406
2.7389	5.5408
2.7604	5.5710
2.7698	5.5801
2.7784	5.5974
2.7790	5.6105
2.7890	5.6236
2.7934	5.6370
2.8086	5.6985
2.8087	5.8100
2.8245	5.9467
2.8364	5.9958
2.8368	6.0004
2.8819	6.0452
2.8859	6.0539
2.8903	6.1027
2.9047	6.1028
2.9213	6.1582
2.9963	6.1893
3.0148	6.2065
3.0523	6.2402
3.0616	6.2494
3.0617	6.3294
3.0942	6.3730
3.0944	6.4478
3.0977	6.4959
3.0999	6.5072
3.1096	6.5674
3.1114	6.5930
3.1177	6.5987
3.1417	6.6004
3.2012	6.6201
3.2057	6.6318
3.2148	6.7132
3.2395	6.7543
3.2438	6.8223
3.2447	6.8387
3.2508	6.8464
3.2559	6.8565
3.2689	6.8739
3.2760	6.9264
3.2777	7.0141
3.2811	7.0627

Table A.11 (cont.)

3.2836	7.0778
3.2853	7.0830
3.2978	7.1463
3.3005	7.1468
3.3329	7.1854
3.3704	7.2079
3.3712	7.2090
3.3842	7.2256
3.3978	7.2424
3.4049	7.2626
3.4168	7.3524
3.4224	7.3621
3.4553	7.4422
3.4933	7.4588
3.4992	7.4828
3.4998	7.7959
3.5016	7.8015
3.5368	7.9435
3.5368	7.9650
3.5673	8.0024
3.5888	8.1036
3.6090	8.2552
3.6193	8.3116
3.6232	8.3172
3.6350	8.3381
3.6462	8.3881
3.6740	8.4837
3.6940	8.5459
3.7717	8.5751
3.8026	8.6463
3.8229	8.7857
3.8357	9.0317
3.8572	9.0340
3.8772	9.0353
3.8929	9.0656
3.9131	9.0777
3.9162	9.1184
3.9231	9.1456
3.9532	9.2164
3.9686	9.5922
3.9903	9.5938
3.9989	9.6924
4.0026	9.7086
4.0203	10.0659
4.0285	10.1122
4.0317	10.1153
4.0412	10.1291
4.0440	10.1633

Table A.11 (cont.)

4.1354	10.1802
4.1475	10.2950
4.1673	10.3745
4.1915	10.4633
4.2179	10.4781
4.2394	10.5118
4.2725	10.5453
4.3018	10.6275
4.3203	10.6592
4.3234	10.7448
4.3382	10.7967
4.3638	11.1933
4.3732	11.1972
4.3914	11.2995
4.4292	11.3569
4.4393	11.5962
4.4634	11.6488
4.4667	11.7100
4.4875	11.7431
4.4993	11.9001
4.5329	11.9640
4.5668	11.9897
4.5850	12.0546
4.5918	12.2516
4.6086	12.2872
4.6147	12.3182
4.6237	12.5060
4.6326	12.5167
4.6935	12.5381
4.7034	12.5785
4.7061	12.6341
4.7129	12.8904
4.7176	13.0381
4.7655	13.0492
4.8007	13.2523
4.8161	13.3335
4.8291	13.4497
4.8767	13.5695
4.8829	13.6334
4.8842	13.6779
4.9137	13.7210
4.9383	13.7398
4.9466	13.7555
4.9684	13.8976
4.9975	14.0340
5.0331	14.1770
5.0483	14.3703
5.0854	14.4555

Table A.11 (cont.)

5.1120	14.6482
5.1262	14.6940
5.1311	15.0658
5.1472	15.1198
5.1479	15.4251
5.1534	15.7202
5.1929	15.7233
5.2054	16.0873
5.2057	16.1318
5.2071	16.1671
5.2311	16.3296
5.2357	16.6064
5.2672	16.6936
5.3232	16.9481
5.3354	17.3029
5.3554	17.5781
5.3746	18.2612
5.3914	18.5327
5.4117	19.0938
5.4149	19.1463
5.4762	19.3918
5.4805	22.0800
5.4858	22.1791
5.4928	22.2330
5.5157	22.4747
5.5316	22.9816
5.5440	23.5616
5.5490	25.0111
5.5577	28.1374
5.6226	35.1124
5.6371	68.9895
5.6419	
5.6669	
5.6784	
5.6999	
5.7440	
5.7498	
5.7609	
5.8070	
5.8170	
5.8223	
5.8463	
5.8551	
5.8665	
5.8949	
5.9085	
5.9798	
6.0014	

Table A.11 (cont.)

6.0198
6.0214
6.0235
6.0603
6.0671
6.1157
6.1292
6.1764
6.1973
6.2021
6.2053
6.2233
6.2312
6.2772
6.2966
6.3026
6.3077
6.3243
6.3464
6.3504
6.3517
6.3862
6.3880
6.4079
6.4435
6.4817
6.5426
6.5558
6.6109
6.6167
6.6358
6.6359
6.6454
6.7184
6.7187
6.7534
6.8975
6.8992
6.9209
6.9479
6.9482
7.0203
7.0359
7.0471
7.0519
7.0562
7.0644
7.0648

Table A.11 (cont.)

7.0902
7.0953
7.1040
7.1575
7.2032
7.2250
7.2326
7.2604
7.3186
7.3273
7.3456
7.3457
7.3944
7.4519
7.4660
7.4863
7.5018
7.5986
7.6758
7.6763
7.7029
7.7171
7.7283
7.7325
7.7467
7.7765
7.7873
7.8338
7.8431
7.8509
7.8690
7.8836
7.8853
7.8953
7.9179
7.9506
7.9508
7.9550
7.9709
8.0170
8.0355
8.0519
8.0539
8.1077
8.1267
8.1579
8.1606
8.1785

Table A.11 (cont.)

8.1843
8.2056
8.2085
8.2218
8.2904
8.2930
8.3355
8.3399
8.3498
8.3518
8.3601
8.3969
8.4565
8.5076
8.5079
8.5194
8.5276
8.5390
8.5543
8.5588
8.6260
8.6612
8.6698
8.6744
8.6998
8.7408
8.8021
8.8095
8.8128
8.8418
8.8591
8.9547
8.9664
8.9882
9.0436
9.0800
9.1510
9.2577
9.2679
9.2785
9.3334
9.3344
9.3439
9.3547
9.4076
9.5336
9.6298
9.6390

Table A.11 (cont.)

9.6540
9.7292
9.7312
9.7337
9.7425
10.0337
10.0518
10.1640
10.1890
10.1920
10.2021
10.2832
10.3461
10.3525
10.4202
10.4311
10.4476
10.6500
10.6678
10.6811
10.6850
10.7367
10.7889
10.8111
10.8310
10.8642
10.9446
10.9801
11.0483
11.0562
11.0899
11.1606
11.2285
11.2553
11.2827
11.2891
11.2950
11.3243
11.3633
11.3773
11.4746
11.4944
11.5434
11.5634
11.5908
11.6036
11.6500
11.6654

Table A.11 (cont.)

11.6737
11.7726
11.8362
11.8775
11.9270
11.9931
12.0015
12.2321
12.3155
12.4833
12.6200
12.6278
12.7569
12.7864
12.9123
12.9786
12.9943
13.0251
13.1433
13.2326
13.4660
13.4668
13.5709
13.6365
13.8232
13.8561
13.8794
13.8951
14.1355
14.1636
14.1974
14.2584
14.3132
14.3401
14.3460
14.3597
14.3891
14.4258
14.4619
14.9068
14.9422
15.0290
15.0692
15.1560
15.3679
15.6029
15.6395
15.6807

Table A.11 (cont.)

15.8719
15.9785
16.0323
16.1623
16.1685
16.2551
16.2683
16.3878
16.5679
16.6169
16.6604
17.1338
17.3493
17.5931
18.0474
18.3872
18.4649
18.6098
18.6949
18.9007
18.9745
19.1213
19.7786
20.2280
20.6263
20.7271
22.0765
22.7155
22.7270
23.5302
23.6932
23.8791
24.4230
25.6618
28.6822
29.8110
30.6622
32.4396
36.9139
44.6625
52.3595
66.1187
71.6317

APPENDIX B

APPENDICES FOR CHAPTER 3

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- Table B.2: Raw data of CHO cell acute cytotoxicity after 4 hours of treatment with chloroacetonitrile (CAN), bromoacetonitrile (BAN) and iodoacetonitrile (IAN).
- Table B.3: Regression analysis of the CHO cell CAN SCGE genotoxicity data.
- Table B.4: Regression analysis of the CHO cell BAN SCGE genotoxicity data.
- Table B.5: Regression analysis of the CHO cell IAN SCGE genotoxicity data.
- Table B.6: Raw data of the DNA repair kinetics in CHO cells treated with CAN (1200 µM), BAN (60 µM) and IAN (60 µM).
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- Table B.8: CHO cell DNA repair kinetics data normalized to the concurrent 0-time control.
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- Table B.12: Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with CAN without and with 1, 2, 3, 4, 5, 10 and 24 hour liquid-holding recovery.

- Table B.13: Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with BAN without and with 1, 2, 3, 4, 5, 10 and 24 hour liquid-holding recovery.
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- Table B.15: Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from the CHO cell negative controls at 0 and 24 hour liquid-holding recovery.
- Table B.16: Raw data of the plating efficiency of HAA-treated CHO cells.
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- Table B.18: Pearson correlation analysis of physicochemical and toxicological measurements of monohaloacetonitriles.

Table B.1 Raw data of CHO cell genomic DNA damage after 4 hours of treatment with chloroacetonitrile (CAN), bromoacetonitrile (BAN) and iodoacetonitrile (IAN). The primary measure of DNA damage is the percent of the DNA that had migrated from the nucleus into the microgel (% tail DNA). Twenty-five randomly chosen nuclei per microgel were analyzed and the % tail DNA values were averaged to represent the microgel.

	Expt 061511ykCAN		Expt 061711ykCAN		Expt 062011ykCAN		num	Average	SE[a]	SD[b]
CAN conc. (μM)	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B				
0	3.64272	1.96116	1.74276	1.21191	1.41049	1.98830	6	1.99289	0.353	0.864
100	2.21754	4.37266	2.86132	6.10492	2.32559	1.92984	6	3.30198	0.664	1.625
400	22.0987	23.7556	28.5017	10.1226	21.4516	29.4387	6	22.5615	2.829	6.931
600	35.3997	23.8242	22.7507	17.8022	40.5908	31.9305	6	28.7164	3.534	8.656
800	36.1930	36.4094	37.9336	32.5197	43.7722	35.5994	6	37.0712	1.525	3.735
900	43.7084	46.4054					2	45.0569	1.349	1.907
1000	42.4778	34.8863	67.4607	57.3985	65.4046	61.7428	6	54.8951	5.404	13.237
1200	60.5293	48.9537	65.0847	62.9409	64.8041	62.9788	6	60.8819	2.477	6.068
1400	67.6226	71.5714	69.5695	67.1266	54.8826	65.4780	6	66.0418	2.391	5.857
1600	74.7260		71.1449	66.8572	64.0812	57.0839	5	66.7786	3.029	6.773
1800			67.5688	67.8832	72.0506	71.4590	4	69.7404	1.171	2.342
2000			65.4860		77.6190		2	71.5525	6.066	8.579

	Expt 051811ykBAN		Expt 052311ykBAN		Expt 060111ykBAN		num	Average	SE[a]	SD[b]
BAN conc. (μM)	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B				
0	1.95989	3.95020	1.61536	2.81502	3.54138	4.42749	6	3.05156	0.456	1.118
10	0.951737	2.60152	4.63930	2.20560	2.20390	2.82873	6	2.57180	0.491	1.203
20	4.73189	7.29887	9.68580	4.02349	3.26640	2.80745	6	5.30232	1.088	2.666
40	25.1729	9.2209	28.9393	20.1097	11.0390	13.7974	6	18.0466	3.260	7.984
50	24.0237	19.8509	39.3131	51.5661	17.3446	26.6842	6	29.7971	5.359	13.126
55	45.1792	43.8633	63.9535	60.8512	51.0122	46.5937	6	51.9088	3.484	8.534
60	61.2937	56.6847	63.8626	57.8668	48.2192	55.2422	6	57.1949	2.209	5.410
65	61.6118	67.0802	84.6619	85.1150	76.7127	77.5453	6	75.4545	3.855	9.443
70	82.3306	85.6862	92.5099	91.0710	86.4037	84.5830	6	87.0974	1.598	3.914
80	88.9635	93.2285	94.4138	92.4375	91.9199	92.5733	6	92.2561	0.746	1.827
100	96.2988		95.4813		91.3766		3	94.3855	1.523	2.638

	Expt 051811ykIAN		Expt 052311ykIAN		Expt 060111ykIAN		num	Average	SE[a]	SD[b]
IAN conc. (μM)	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B				
0	2.52942	1.01246	2.18911	1.75668	3.65528	0.82735	6	1.99505	0.427	1.046
10	2.32128	0.953169	2.05322	3.33821	3.46340	2.24202	6	2.39522	0.377	0.923
30	8.19238	3.75402	7.92972	9.77290	3.26403	3.67707	6	6.09835	1.164	2.851
40	4.56923	4.76666	12.5772	8.66238	11.4164	16.4002	6	9.73201	1.896	4.644
45	7.48082	8.24485	20.6323	19.1626	19.5076	21.0789	6	16.0178	2.597	6.360
50	14.4175	15.9381	42.6844	41.6367	44.8613	49.3393	6	34.8129	6.305	15.445
55	36.5539	39.4266	45.1948	46.8895	65.3303	54.3606	6	47.9593	4.299	10.530
60	52.4345	50.2757	50.7337	41.6187	75.7784	73.6176	6	57.4097	5.685	13.926
65	75.2622	69.1382	73.7079	83.1950	94.0035	89.0538	6	80.7268	3.938	9.645
70			93.6779	91.5105	93.0830	90.6627	4	92.2335	0.695	1.390
75			85.0621		91.3174		2	88.1898	3.128	4.423

[a]SE: standard error of the mean

[b]SD: standard deviation

Table B.2 Raw data of CHO cell acute cytotoxicity after 4 hours of treatment with chloroacetonitrile (CAN), bromoacetonitrile (BAN) and iodoacetonitrile (IAN). Acute cytotoxicity was analyzed with the trypan blue dye exclusion assay.

	061511ykCAN		061711ykCAN		062011ykCAN		Acute cytotoxicity total		
CAN conc. (µM)	Live	Dead	Live	Dead	Live	Dead	Live	Dead	% Cell viability
0	128	6	137	10	144	8	409	24	94.46
100	145	10	156	11	107	4	408	25	94.23
400	147	4	134	3	112	10	393	17	95.85
600	210	3	106	6	156	19	472	28	94.40
800	178	5	115	4	135	4	428	13	97.05
900	123	1					123	1	99.19
1000	154	1	132	6	116	3	402	10	97.57
1200	122	2	129	4	120	3	371	9	97.63
1400	125	4	129	4	141	0	395	8	98.01
1600	182	1	131	1	146	4	459	6	98.71
1800			155	4	155	0	310	4	98.73
2000			204	0	146	1	350	1	99.72

	051811ykBAN		052311ykBAN		060111ykBAN		Acute cytotoxicity total		
BAN conc. (µM)	Live	Dead	Live	Dead	Live	Dead	Live	Dead	% Cell viability
0	129	2	211	21	137	5	477	28	94.46
10	144	5	143	8	149	14	436	27	94.17
20	121	3	111	6	146	12	378	21	94.74
40	121	4	145	10	127	4	393	18	95.62
50	168	3	103	4	146	8	417	15	96.53
55	152	5	150	1	117	5	419	11	97.44
60	162	3	172	6	154	9	488	18	96.44
65	145	2	152	4	151	6	448	12	97.39
70	127	3	171	12	166	0	464	15	96.87
80	164	5	180	5	109	1	453	11	97.63
100	139	5	139	3	105	4	383	12	96.96

	051811ykIAN		052311ykIAN		060111ykIAN		Acute cytotoxicity total		
IAN conc. (µM)	Live	Dead	Live	Dead	Live	Dead	Live	Dead	% Cell viability
0	164	6	113	1	206	20	483	27	94.71
10	142	15	130	6	137	10	409	31	92.95
30	110	0	120	3	149	4	379	7	98.19
40	143	4	137	9	139	7	419	20	95.44
45	126	3	127	3	144	8	397	14	96.59
50	140	9	143	8	140	1	423	18	95.92
55	117	1	125	3	135	5	377	9	97.67
60	153	14	154	7	133	3	440	24	94.83
65	138	1	131	6	159	8	428	15	96.61
70			137	6	152	4	289	10	96.66
75			182	7	149	5	331	12	96.50

Table B.3 Regression analysis of the CHO cell CAN SCGE genotoxicity data.

Nonlinear Regression **Wednesday, July 25, 2012, 9:00:21 AM**

Data Source: all HANs_3 repeats each in Redo2_haloacetonitriles
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9943	0.9886	0.9861	2.9705

	Coefficient	Std. Error	t	P
a	71.2863	2.0241	35.2181	<0.0001
b	277.6382	27.6349	10.0467	<0.0001
x0	721.5647	30.5139	23.6471	<0.0001

Analysis of Variance:

Analysis of Variance:

	DF	SS	MS
Regression	3	30197.9701	10065.9900
Residual	9	79.4150	8.8239
Total	12	30277.3851	2523.1154

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	6913.9249	3456.9624	391.7731	<0.0001
Residual	9	79.4150	8.8239		
Total	11	6993.3399	635.7582		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.4356)

W Statistic= 0.9350 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0113)

Fit Equation Description:

```
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 71.2863}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 277.638}}
x0 = x50(x,y,.5) "Auto {{previous: 721.565}}
```

Table B.3 (cont.)

```
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 7
```

Table B.4 Regression analysis of the CHO cell BAN SCGE genotoxicity data.

Nonlinear Regression **Wednesday, July 25, 2012, 9:04:56 AM**

Data Source: all HANs_3 repeats each in Redo2_haloacetonitriles
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9953	0.9906	0.9883	3.9996

	Coefficient	Std. Error	t	P
a	97.2262	3.4759	27.9711	<0.0001
b	8.5454	0.9990	8.5536	<0.0001
x0	55.0910	1.0581	52.0653	<0.0001

Analysis of Variance:

Analysis of Variance:

	DF	SS	MS
Regression	3	37794.5422	12598.1807
Residual	8	127.9763	15.9970
Total	11	37922.5185	3447.5017

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	13489.2854	6744.6427	421.6182	<0.0001
Residual	8	127.9763	15.9970		
Total	10	13617.2617	1361.7262		

Statistical Tests:

Normality Test (Shapiro-Wilk) Failed (P = 0.0407)

W Statistic= 0.8484 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.6138)

Fit Equation Description:

```
[Variables]
x = col(6)
y = col(7)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 97.2262}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 8.54539}}
x0 = x50(x,y,.5) "Auto {{previous: 55.091}}
```

Table B.4 (cont.)

```
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 9
```

Table B.5 Regression analysis for the CHO cell IAN SCGE genotoxicity data.

Nonlinear Regression **Wednesday, July 25, 2012, 9:07:26 AM**

Data Source: all HANs_3 repeats each in Redo2_haloacetonitriles
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9945	0.9890	0.9862	4.1667

	Coefficient	Std. Error	t	P
a	99.3082	6.3677	15.5957	<0.0001
b	7.3274	0.9987	7.3367	<0.0001
x0	55.7228	1.4296	38.9766	<0.0001

Analysis of Variance:

Analysis of Variance:

	DF	SS	MS
Regression	3	29868.4758	9956.1586
Residual	8	138.8890	17.3611
Total	11	30007.3648	2727.9423

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	12462.3047	6231.1524	358.9141	<0.0001
Residual	8	138.8890	17.3611		
Total	10	12601.1937	1260.1194		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.3629)

W Statistic= 0.9250 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0209)

Fit Equation Description:

```
[Variables]
x = col(11)
y = col(12)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 99.3082}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 7.32744}}
x0 = x50(x,y,.5) "Auto {{previous: 55.7228}}
```

Table B.5 (cont.)

```
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 8
```

Table B.6 Raw data of the DNA repair kinetics in CHO cells treated with CAN (1200 µM), BAN (60 µM) and IAN (60 µM). CHO cells were treated for 4 hours and then maintained in fresh serum-free F12 medium for 0, 1, 2, 3, 4, 5, 10 and 24 hours (liquid holding (LH) recovery time). The primary measure of DNA damage is the percent of the DNA that had migrated from the nucleus into the microgel (% tail DNA). Fifty randomly chosen nuclei per microgel were analyzed and the % tail DNA values were averaged to represent the microgel.

		Expt 062011ykCAN		Expt 062511ykCAN		Expt 062711ykCAN		Expt 091611ykCAN1		Expt 091611ykCAN2	
CAN conc. (µM)	LH time (hours)	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B
0	0	1.44555	5.36062	2.36573	2.16841	2.23912	3.05889	1.12785	2.67704	1.44553	0.948998
1200	0	66.4780	67.5452	71.4820	70.8790	53.2424	50.5131	86.6393	80.4939	76.1991	78.7079
1200	1	40.1499	33.9285	31.9256	27.7707	29.0436	31.0792	40.5000	30.8236	31.7320	35.1518
1200	2	18.6537	17.7883	16.7622	15.1922	11.5711	10.3637	15.0196	17.5154	21.7027	17.0114
1200	3	7.68463	9.12812	7.21894	10.2309	7.22534	10.1502	13.0875	13.6518	16.7490	15.2385
1200	4	6.29296	7.23056	6.46178	5.76068	3.69305	11.2429	16.6516	14.6841	11.7075	9.14558
1200	5	5.01604	5.18677	6.32143	6.09288	9.51049	8.61297	7.25956	10.0845	21.7171	21.1795
1200	10	4.48517	6.94614	6.10198	6.02602	2.48453	3.46302	4.98485	5.84498	3.36851	2.73555
0	24	1.38544	2.38646	2.13149	1.74355	1.22911	2.70744	1.48126	0.81807	2.74775	1.12390
1200	24	6.85333	9.08027	2.12212	5.40813	6.08648	9.10647	4.57900	6.37228	5.70985	6.79627

		Expt 090111ykBAN1		Expt 090111ykBAN2		Expt 122811ykBAN1		Expt 122811ykBAN2	
BAN conc. (µM)	LH time (hours)	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B
0	0	1.25966	0.993854	1.40884	0.922386	2.86008	0.579878	3.97513	0.450705
60	0	63.3073	65.2709	61.3408	63.5231	82.7047	78.5670	84.9747	81.3419
60	1	33.1644	28.6729	35.4852	31.4976	50.3471	39.6332	59.9963	59.9507
60	2	18.0949	19.2297	23.5586	27.0931	39.7012	39.9257	36.1382	49.7377
60	3	15.7960	7.51916	11.7051	13.0849	43.5516	38.0450	21.9558	26.7007
60	4	14.9173	7.51447	10.6069	10.1366	18.4730	16.7659	32.6840	27.8108
60	5	10.4311	7.81101	8.72176	7.18749	18.3776	14.2613	13.3392	15.2156
60	10	5.55173	2.91165	3.89964	6.96668	9.14963	12.8597	10.1313	8.73536
0	24	2.65816	0.728611	1.54300	0.621775	1.88938	1.03530	0.922628	0.789595
60	24	4.44810	4.64079	3.98455	5.50343	10.2206	8.37669	8.52657	8.56215

		Expt 080111ykIAN1		Expt 080111ykIAN2		Expt 092411ykIAN1		Expt 092411ykIAN2	
IAN conc. (µM)	LH time (hours)	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B
0	0	2.32416	2.74211	1.93535	2.27058	1.31850	0.523633	1.10125	1.53764
60	0	65.2649	55.7417	64.0777	57.3944	60.2983	55.3135	66.6261	64.1978
60	1	38.6554	36.9702	41.9454	40.1165	22.2427	16.9448	23.0777	13.3046
60	2	35.5034	35.9433	39.0028	39.0157	23.5421	16.9527	25.3428	22.2191
60	3	16.0701	12.4914	22.7355	14.3433	12.8090	7.4747	22.3749	14.8885
60	4	7.64693	9.03924	13.7703	10.7907	12.7986	11.7656	11.2958	8.67954
60	5	6.34129	7.57376	16.3207	14.1220	11.2036	14.2469	10.9556	12.6269
60	10	6.50883	6.84642	6.86923	8.22927	5.68660	4.67269	5.45514	7.45114
0	24	1.25125	1.74059	1.01518	3.04596	0.294099	0.791296	0.791629	0.463279
60	24	7.01616	8.79110	6.73367	9.09013	7.47604	4.34950	6.26346	6.61167

Table B.7 Raw data of the percent viability of CHO cells during the DNA repair kinetics after treated with CAN (1200 µM), BAN (60 µM) and IAN (60 µM). CHO cells were treated for 4 hours and then maintained in fresh serum-free F12 medium for 0, 1, 2, 3, 4, 5, 10 and 24 hours (liquid holding (LH) recovery time).

		062011ykCAN		062511ykCAN		062711ykCAN		091611ykCAN1		091611ykCAN2		Acute cytotoxicity total		
CAN conc. (µM)	LH time (hours)	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	% Cell viability
0	0	144	4	228	12	213	9	163	7	153	2	901	34	96.36
1200	0	223	9	147	7	155	5	187	8	202	5	914	34	96.41
1200	1	177	8	241	8	267	5	192	2	198	5	1075	28	97.46
1200	2	192	5	183	2	200	1	166	2	139	2	880	12	98.65
1200	3	214	1	277	3	174	4	173	13	192	4	1030	25	97.63
1200	4	195	2	184	11	139	6	200	0	196	6	914	25	97.34
1200	5	201	1	262	8	229	6	139	2	122	1	953	18	98.15
1200	10	122	20	225	5	186	6	138	7	145	4	816	42	95.10
0	24	206	5	243	7	225	14	169	18	174	3	1017	47	95.58
1200	24	169	13	231	3	139	22	150	13	178	22	867	73	92.23

		090111ykBAN1		090111ykBAN2		122811ykBAN1		122811ykBAN2		Acute cytotoxicity total		
BAN conc. (µM)	LH time (hours)	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	% Cell viability
0	0	171	3	137	10	104	3	117	0	529	16	97.06
60	0	172	10	158	7	183	4	108	3	621	24	96.28
60	1	161	1	146	0	254	3	171	1	732	5	99.32
60	2	149	3	147	2	220	6	147	1	663	12	98.22
60	3			189	6	157	6	100	2	446	14	96.96
60	4	159	4	174	3	102	1	106	1	541	9	98.36
60	5	200	1	150	3	143	4	228	7	721	15	97.96
60	10	244	9	163	3	129	4	108	1	644	17	97.43
0	24	190	18	139	5	117	4	146	7	592	34	94.57
60	24	173	8	153	10	160	5	146	11	632	34	94.89

		080111ykIAN1		080111ykIAN2		092411ykIAN1		092411ykIAN2		Acute cytotoxicity total		
IAN conc. (µM)	LH time (hours)	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	% Cell viability
0	0	172	11	122	4	171	11	211	9	676	35	95.08
60	0	151	2	142	3	131	0	210	5	634	10	98.45
60	1	191	10	143	2	153	4	211	6	698	22	96.94
60	2	125	1	150	9	202	3	137	3	614	16	97.46
60	3	158	4	184	3	152	0	157	3	651	10	98.49
60	4	196	6	137	2	185	8	196	1	714	17	97.67
60	5	158	4	170	5	172	4	142	4	642	17	97.42
60	10	183	8	169	2	121	1	131	4	604	15	97.58
0	24	198	21	131	6	179	12	175	10	683	49	93.31
60	24	136	6	192	13	159	5	152	23	639	47	93.15

Table B.8 CHO cell DNA repair kinetics data normalized to the concurrent 0-time control (%).

CAN 1200 µM

	062011ykCAN		062511ykCAN		062711ykCAN		091611ykCAN1		091611ykCAN2		num	Average	SE[a]	SD[b]
LH time (hours)	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B				
0	99.20	100.80	100.42	99.58	102.63	97.37	103.68	96.32	98.38	101.62	10	100.00	0.728	2.303
1	59.91	50.63	44.85	39.01	55.98	59.91	48.46	36.89	40.97	45.38	10	48.20	2.635	8.332
2	27.84	26.55	23.55	21.34	22.30	19.98	17.97	20.96	28.02	21.96	10	23.05	1.077	3.404
3	11.47	13.62	10.14	14.37	13.93	19.57	15.66	16.34	21.62	19.67	10	15.64	1.175	3.715
4	9.39	10.79	9.08	8.09	7.12	21.67	19.93	17.57	15.12	11.81	10	13.06	1.639	5.184
5	7.49	7.74	8.88	8.56	18.33	16.60	8.69	12.07	28.04	27.34	10	14.37	2.514	7.949
10	6.69	10.37	8.57	8.47	4.79	6.68	5.97	6.99	4.35	3.53	10	6.64	0.666	2.105
24	10.23	13.55	2.98	7.60	11.73	17.55	5.48	7.63	7.37	8.77	10	9.29	1.322	4.179

BAN 60 µM

	090111ykBAN1		090111ykBAN2		122811ykBAN1		122811ykBAN2		num	Average	SE[a]	SD[b]
LH time (hours)	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B				
0	98.47	101.53	98.25	101.75	102.57	97.43	102.18	97.82	8	100.00	0.773	2.187
1	51.59	44.60	56.84	50.45	62.44	49.15	72.15	72.09	8	57.41	3.715	10.509
2	28.15	29.91	37.73	43.40	49.24	49.51	43.46	59.81	8	42.65	3.739	10.576
3	24.57	11.70	18.75	20.96	54.01	47.18	26.40	32.11	8	29.46	5.105	14.440
4	23.20	11.69	16.99	16.24	22.91	20.79	39.30	33.44	8	23.07	3.245	9.177
5	16.23	12.15	13.97	11.51	22.79	17.69	16.04	18.30	8	16.08	1.291	3.651
10	8.64	4.53	6.25	11.16	11.35	15.95	12.18	10.50	8	10.07	1.264	3.574
24	6.92	7.22	6.38	8.82	12.68	10.39	10.25	10.30	8	9.12	0.767	2.170

IAN 60 µM

	080111ykIAN1		080111ykIAN2		092411ykIAN1		092411ykIAN2		num	Average	SE[a]	SD[b]
LH time (hours)	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B				
0	107.87	92.13	105.50	94.50	104.31	95.69	101.86	98.14	8	100.00	2.020	5.713
1	63.89	61.10	69.06	66.05	38.48	29.31	35.28	20.34	8	47.94	6.762	19.125
2	58.68	59.41	64.22	64.24	40.73	29.33	38.74	33.97	8	48.66	5.091	14.400
3	26.56	20.65	37.43	23.62	22.16	12.93	34.21	22.76	8	25.04	2.743	7.759
4	12.64	14.94	22.67	17.77	22.14	20.35	17.27	13.27	8	17.63	1.366	3.864
5	10.48	12.52	26.87	23.25	19.38	24.65	16.75	19.30	8	19.15	2.034	5.752
10	10.76	11.32	11.31	13.55	9.84	8.08	8.34	11.39	8	10.57	0.632	1.788
24	11.60	14.53	11.09	14.97	12.93	7.52	9.58	10.11	8	11.54	0.895	2.532

[a]SE: standard error of the mean

[b]SD: standard deviation

Table B.9 Regression analysis of the CHO cell CAN DNA repair kinetics data.

Nonlinear Regression **Wednesday, July 25, 2012, 10:07:14 AM**
CAN repair kinetics

Data Source: All HANs as % in Redo2_haloacetonitriles
Equation: Exponential Decay, Single, 3 Parameter
f=y0+a*exp(-b*x)

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9979	0.9959	0.9942	2.3977

	Coefficient	Std. Error	t	P
y0	9.5264	1.2849	7.4139	0.0007
a	90.6220	2.6065	34.7683	<0.0001
b	0.8804	0.0611	14.4002	<0.0001

Analysis of Variance:

Analysis of Variance:

	DF	SS	MS
Regression	3	13577.7987	4525.9329
Residual	5	28.7457	5.7491
Total	8	13606.5444	1700.8180

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	6951.0710	3475.5355	604.5319	<0.0001
Residual	5	28.7457	5.7491		
Total	7	6979.8167	997.1167		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.7256)

W Statistic= 0.9514 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.4597)

Fit Equation Description:

```
[Variables]
x = col(1)
y = col(2)
reciprocal_y=1/abs(y)
reciprocal_ysquare=1/y^2
'Automatic Initial Parameter Estimates
yhat(q)=q[size(q)]
[Parameters]
y0 = yhat(y) "Auto {{previous: 9.52641}}
a = max(y)-yhat(y) "Auto {{previous: 90.622}}
b = if(x50(x,y-yhat(y))=0, 1,  -ln(.5)/x50(x,y-yhat(y))) "Auto {{previous: 0.880392}}
```

Table B.9 (cont.)

```
[Equation]
f=y0+a*exp(-b*x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
b>0
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 8
```

Table B.10 Regression analysis of the CHO cell BAN DNA repair kinetics data.

Nonlinear Regression **Wednesday, July 25, 2012, 10:08:38 AM**
BAN repair kinetics

Data Source: All HANs as % in Redo2_haloacetonitriles
Equation: Exponential Decay, Single, 3 Parameter
f=y0+a*exp(-b*x)

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9972	0.9944	0.9922	2.7070

	Coefficient	Std. Error	t	P
y0	10.1848	1.7892	5.6925	0.0023
a	88.0792	2.9438	29.9200	<0.0001
b	0.5269	0.0396	13.3043	<0.0001

Analysis of Variance:

Analysis of Variance:

	DF	SS	MS
Regression	3	16922.0240	5640.6747
Residual	5	36.6403	7.3281
Total	8	16958.6643	2119.8330

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	6563.7256	3281.8628	447.8488	<0.0001
Residual	5	36.6403	7.3281		
Total	7	6600.3659	942.9094		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.1071)

W Statistic= 0.8550 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.0588)

Fit Equation Description:

```
[Variables]
x = col(1)
y = col(5)
reciprocal_y=1/abs(y)
reciprocal_ysquare=1/y^2
'Automatic Initial Parameter Estimates
yhat(q)=q[size(q)]
[Parameters]
y0 = yhat(y) "Auto {{previous: 10.1848}}
a = max(y)-yhat(y) "Auto {{previous: 88.0792}}
b = if(x50(x,y-yhat(y))=0, 1,  -ln(.5)/x50(x,y-yhat(y))) "Auto {{previous: 0.526941}}
```

Table B.10 (cont.)

```
[Equation]
f=y0+a*exp(-b*x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
b>0
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 10
```

Table B.11 Regression analysis of the CHO cell IAN DNA repair kinetics data.

Nonlinear Regression **Wednesday, July 25, 2012, 10:10:31 AM**
IAN repair kinetics

Data Source: All HANs as % in Redo2_haloacetonitriles
Equation: Exponential Decay, Single, 3 Parameter
f=y0+a*exp(-b*x)

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9792	0.9588	0.9424	7.2476

	Coefficient	Std. Error	t	P
y0	12.2210	4.5377	2.6932	0.0431
a	84.9370	7.8721	10.7897	0.0001
b	0.6028	0.1276	4.7263	0.0052

Analysis of Variance:

Analysis of Variance:

	DF	SS	MS
Regression	3	15953.2104	5317.7368
Residual	5	262.6370	52.5274
Total	8	16215.8474	2026.9809

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	6115.6101	3057.8050	58.2135	0.0003
Residual	5	262.6370	52.5274		
Total	7	6378.2470	911.1781		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.4856)

W Statistic= 0.9266 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0287)

Fit Equation Description:

```
[Variables]
x = col(1)
y = col(8)
reciprocal_y=1/abs(y)
reciprocal_ysquare=1/y^2
'Automatic Initial Parameter Estimates
yhat(q)=q[size(q)]
[Parameters]
y0 = yhat(y) "Auto {{previous: 12.221}}
a = max(y)-yhat(y) "Auto {{previous: 84.937}}
b = if(x50(x,y-yhat(y))=0, 1,  -ln(.5)/x50(x,y-yhat(y))) "Auto {{previous: 0.602845}}
```

Table B.11 (cont.)

```
[Equation]
f=y0+a*exp(-b*x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
b>0
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 10
```

Table B.12 Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with CAN without and with 1, 2, 3, 4, 5, 10 and 24 hour liquid-holding recovery.

LH_0h	LH_1h	LH_2h	LH_3h	LH_4h	LH_5h	LH_10h	LH_24h
8.82938	0.01858	0	0	0	0	0	0
10.9017	0.03193	0	0	0	0	0	0
13.7598	0.03450	0	0	0	0	0	0
13.7981	0.07327	0	0	0	0	0	0
15.1714	0.09674	0	0	0	0	0	0
15.8492	0.21394	0	0	0	0	0	0
16.4707	0.30928	0	0	0	0	0	0
17.7065	0.34539	0	0	0	0	0	0
17.7073	0.43927	0.001254	0	0	0	0	0
21.0131	0.65040	0.001473	0	0	0	0	0
21.6998	1.18736	0.001607	0	0	0	0	0
23.2550	1.23293	0.002494	0	0	0	0	0
23.7059	1.37611	0.002876	0	0	0	0	0
23.8447	1.46813	0.003321	0.001120	0	0	0	0
25.1772	1.76325	0.004161	0.002977	0	0	0	0
26.1630	1.86586	0.004731	0.002980	0	0	0	0
26.2815	2.23414	0.005383	0.003352	0	0.001184	0	0
26.4795	2.26208	0.008481	0.004300	0	0.001366	0	0
26.9313	2.33154	0.011027	0.004328	0	0.001412	0	0
27.1086	2.76098	0.011836	0.005011	0	0.001779	0	0
27.9400	2.79141	0.013133	0.007274	0	0.002132	0	0
28.4601	3.23850	0.014880	0.008091	0	0.002499	0	0
29.8185	3.41395	0.016499	0.009090	0	0.002942	0	0
30.2477	3.62878	0.020580	0.010410	0.000990	0.003155	0	0
31.6378	3.69868	0.024643	0.011881	0.001007	0.003235	0	0
32.1142	3.79441	0.028666	0.013106	0.001250	0.003872	0	0
32.1509	3.82908	0.029713	0.013988	0.001346	0.004507	0	0
33.7546	3.83886	0.031691	0.015894	0.001392	0.005039	0	0
33.7552	3.90296	0.034566	0.018490	0.003649	0.005696	0	0
33.8864	3.97622	0.035467	0.019052	0.005701	0.005865	0	0
33.9133	4.15951	0.037290	0.019593	0.005711	0.005906	0.000840	0
34.2731	4.22959	0.038790	0.019679	0.006750	0.005981	0.000998	0.000630
34.7368	4.57668	0.048401	0.023605	0.006798	0.006215	0.001212	0.000680
35.0567	4.72165	0.062882	0.027760	0.008280	0.007577	0.001599	0.000718
35.7978	4.82076	0.067338	0.027958	0.008570	0.007871	0.001631	0.001021
35.8472	5.38760	0.069803	0.029456	0.008791	0.008163	0.001649	0.001291
35.8479	5.39760	0.074155	0.030093	0.009413	0.008985	0.002198	0.001422
35.8674	5.46136	0.076736	0.032935	0.010718	0.013717	0.002230	0.001436
36.1362	5.46869	0.077580	0.039820	0.010798	0.014595	0.002253	0.001817
36.7060	5.73003	0.082056	0.040205	0.010962	0.014704	0.002730	0.001913
37.1842	5.80346	0.090323	0.041358	0.011087	0.015085	0.002757	0.002196
37.2228	5.86021	0.098294	0.042603	0.012971	0.016383	0.002845	0.002683
37.3289	6.77239	0.107730	0.057696	0.013119	0.020220	0.003555	0.002966
37.9886	6.85045	0.111494	0.064956	0.013393	0.022603	0.004852	0.003406
38.1561	6.87397	0.116498	0.066024	0.017206	0.028980	0.004911	0.003634

Table B.12 (cont.)

38.5067	6.94888	0.123536	0.067828	0.017880	0.029051	0.005434	0.003703
38.7382	7.32400	0.124134	0.068095	0.018033	0.029164	0.006063	0.003887
38.7489	7.32529	0.131478	0.071547	0.018888	0.031927	0.006369	0.004038
39.1981	7.43299	0.140541	0.074324	0.020176	0.034800	0.007661	0.004105
40.5148	7.67758	0.145163	0.081345	0.021265	0.034962	0.008177	0.004583
40.9521	7.74115	0.145170	0.082731	0.024096	0.038825	0.008980	0.005466
40.9876	7.86423	0.158077	0.086056	0.026137	0.045025	0.009098	0.006201
41.7460	7.90327	0.158515	0.096245	0.026320	0.045034	0.009341	0.006348
41.7529	7.99198	0.193358	0.099712	0.026460	0.046656	0.009373	0.006454
42.0997	8.12956	0.224500	0.103708	0.027750	0.046759	0.009503	0.006578
42.3220	8.24243	0.265414	0.104654	0.027971	0.053761	0.009687	0.007036
42.5762	8.32734	0.296557	0.108749	0.033063	0.058974	0.009848	0.007412
42.7138	8.37929	0.357100	0.114559	0.037242	0.063683	0.010168	0.008044
42.7194	8.38159	0.359331	0.122287	0.042609	0.065370	0.010237	0.008511
42.7979	8.60134	0.385775	0.125753	0.049798	0.067246	0.010570	0.008792
42.8393	8.61148	0.395932	0.129513	0.051880	0.070098	0.010585	0.009106
43.1110	8.76485	0.401359	0.137206	0.051974	0.078626	0.010887	0.009122
43.7123	8.80697	0.647389	0.145030	0.056202	0.085041	0.012091	0.009177
43.7643	8.81398	0.658781	0.146328	0.056652	0.086215	0.012329	0.009411
44.0560	8.93410	0.703718	0.155729	0.057394	0.086784	0.012464	0.010017
44.0911	9.03770	0.710923	0.163983	0.060961	0.087281	0.013860	0.011501
44.7746	9.14882	0.744881	0.167235	0.065791	0.090020	0.013949	0.012268
44.8449	9.17046	0.762845	0.177743	0.071274	0.090115	0.014702	0.013274
44.9723	9.35331	0.812567	0.203109	0.079531	0.091936	0.015206	0.013642
45.0652	9.41023	0.830725	0.220516	0.081525	0.093304	0.017407	0.015139
45.4283	9.41278	0.832298	0.221309	0.085424	0.096850	0.018438	0.015189
46.9474	9.76562	0.835287	0.231365	0.093225	0.099939	0.019933	0.017480
47.1630	9.76758	0.854719	0.280711	0.096234	0.110086	0.020241	0.017493
47.1978	9.83939	0.940883	0.317938	0.097936	0.111654	0.021614	0.018670
48.2389	10.2245	0.981902	0.332861	0.108407	0.115322	0.022088	0.019308
48.8963	10.3444	1.00784	0.340907	0.112189	0.116365	0.022112	0.020299
49.3181	10.3506	1.01606	0.366146	0.112501	0.123238	0.022271	0.020350
49.5204	10.4021	1.03640	0.366702	0.112767	0.123660	0.023751	0.020491
49.8946	10.4512	1.10715	0.383288	0.116228	0.134710	0.023795	0.020757
49.9151	10.4791	1.14206	0.388496	0.121354	0.144448	0.025686	0.023604
49.9439	10.5076	1.22375	0.396647	0.123541	0.144738	0.026129	0.026316
49.9519	10.9575	1.26927	0.399547	0.142181	0.148793	0.026191	0.028526
50.0644	11.1855	1.44124	0.406100	0.150058	0.175400	0.028136	0.030375
50.5234	11.4796	1.45059	0.408419	0.163210	0.211613	0.029807	0.031101
50.7461	11.5372	1.46967	0.444174	0.172816	0.216554	0.030444	0.031734
51.2931	11.6517	1.47073	0.454146	0.174443	0.228375	0.030698	0.033671
51.2968	11.8406	1.47779	0.496427	0.180185	0.229993	0.032840	0.034140
51.4993	12.2337	1.50750	0.542019	0.181518	0.234327	0.036204	0.035409
51.9041	12.2680	1.52077	0.562173	0.184457	0.240244	0.036567	0.036675
52.0529	12.3771	1.52497	0.565367	0.189411	0.242019	0.038500	0.037653
52.0676	12.4169	1.57500	0.601898	0.192286	0.246223	0.041335	0.041652
52.0814	12.5057	1.61120	0.650242	0.203028	0.247392	0.041355	0.043615
52.1712	12.5253	1.62939	0.700260	0.206466	0.263252	0.043385	0.046713

Table B.12 (cont.)

52.3771	12.6347	1.62944	0.702405	0.220243	0.265833	0.046704	0.047314
52.4679	12.9288	1.63773	0.733384	0.221568	0.268417	0.047922	0.047324
52.4906	13.0706	1.66566	0.768771	0.224911	0.285978	0.051924	0.047943
52.5389	13.1566	1.66670	0.825086	0.227406	0.286268	0.052374	0.048778
52.5818	13.2941	1.66903	0.828516	0.228736	0.290663	0.053535	0.053515
52.7168	13.4582	1.67400	0.858265	0.237770	0.301743	0.056978	0.054232
52.7230	13.8212	1.79166	0.874587	0.250393	0.332979	0.063382	0.056450
52.8198	13.8858	1.86741	0.882367	0.255441	0.343440	0.066418	0.057220
52.8384	13.9821	1.95906	0.889590	0.263091	0.354470	0.069402	0.057393
53.0343	14.0569	2.05134	0.890484	0.268094	0.357023	0.069495	0.057415
53.0447	14.1944	2.05579	0.893097	0.278604	0.362042	0.072413	0.057967
53.1288	14.2125	2.08522	0.903709	0.284278	0.384731	0.073024	0.059571
53.1867	14.2676	2.20411	0.938567	0.286198	0.395026	0.074552	0.061244
53.5474	14.3499	2.27036	0.955665	0.303728	0.397526	0.074924	0.063586
53.5893	14.4367	2.28758	0.959120	0.322887	0.404715	0.075334	0.064536
53.7703	14.6137	2.31567	1.00988	0.331001	0.406767	0.078775	0.065172
53.9162	14.7078	2.32606	1.01537	0.333973	0.413232	0.079735	0.066900
54.0225	14.7703	2.39998	1.01722	0.340172	0.417242	0.080667	0.069033
54.1181	14.9141	2.42125	1.10270	0.346085	0.433320	0.081765	0.071573
54.1793	14.9210	2.44362	1.10468	0.347110	0.435180	0.086645	0.073627
54.4440	15.1134	2.46992	1.10731	0.377804	0.437868	0.101187	0.075456
54.4899	15.1788	2.59462	1.11382	0.383070	0.447413	0.104909	0.075695
54.5037	15.2851	2.61256	1.15967	0.388375	0.459613	0.105710	0.076043
54.5418	15.3023	2.62396	1.16529	0.399232	0.479777	0.113361	0.076537
54.6413	15.3163	2.64680	1.18324	0.408307	0.495806	0.115413	0.077403
55.1287	15.3918	2.66087	1.22006	0.439558	0.497581	0.121667	0.077735
55.2076	15.5270	2.74062	1.31514	0.439696	0.505222	0.130189	0.081582
55.5718	15.6599	2.79050	1.33460	0.448232	0.524016	0.131474	0.089324
55.7837	15.7400	2.81749	1.36470	0.494152	0.547157	0.132574	0.090703
55.9326	15.7883	2.95515	1.37191	0.495591	0.575993	0.139080	0.091909
55.9842	15.9590	3.03823	1.37706	0.498119	0.582786	0.149030	0.095643
56.2348	16.0786	3.05191	1.38202	0.518400	0.589240	0.149523	0.099369
56.2730	16.2455	3.08933	1.38371	0.529500	0.622143	0.152545	0.108248
56.3584	16.3388	3.12333	1.44069	0.534819	0.628108	0.156135	0.115940
56.5145	16.4714	3.18066	1.46023	0.546566	0.635413	0.160328	0.122509
56.5156	16.6406	3.19027	1.47992	0.554012	0.642036	0.160448	0.128541
56.7029	16.6719	3.22308	1.48379	0.580536	0.644039	0.162352	0.139588
56.7701	16.7599	3.22813	1.51165	0.591562	0.649859	0.174692	0.153077
56.8316	16.9098	3.26855	1.51618	0.609817	0.654476	0.175033	0.157611
56.8822	17.0334	3.27624	1.57957	0.616879	0.687530	0.175962	0.158504
57.1168	17.1549	3.35484	1.58221	0.623790	0.700269	0.180026	0.159437
57.3943	17.1561	3.56760	1.59560	0.647992	0.707369	0.185428	0.169298
57.4216	17.1893	3.63098	1.62251	0.652349	0.711259	0.194163	0.170822
57.5013	17.2419	3.65130	1.62895	0.655616	0.718846	0.203303	0.173411
57.6462	17.5159	3.65989	1.62917	0.689452	0.754673	0.205860	0.177680
57.7979	17.5167	3.66915	1.70887	0.712063	0.764217	0.207790	0.179635
57.8419	17.6994	3.67775	1.80182	0.719514	0.771573	0.209925	0.195777
58.4156	17.9041	3.71352	1.84793	0.790800	0.771719	0.213905	0.197379

Table B.12 (cont.)

58.5188	18.1593	3.72593	1.85169	0.791434	0.786516	0.225702	0.198080
58.7802	18.2606	3.88851	1.92291	0.811480	0.809016	0.230833	0.203148
58.8057	18.3225	3.89606	2.00906	0.819986	0.829319	0.231504	0.209525
58.8566	18.3686	3.96753	2.01410	0.851247	0.834300	0.236037	0.214728
58.8805	18.3775	4.04982	2.04669	0.851708	0.858802	0.237025	0.225854
59.1518	18.7958	4.05384	2.04826	0.867576	0.875441	0.246774	0.228863
59.4667	18.8755	4.14483	2.11393	0.882220	0.911490	0.249561	0.236907
59.4946	18.8772	4.15018	2.12153	0.893785	0.950211	0.249675	0.238084
59.5868	18.9146	4.16955	2.13407	0.923049	0.950821	0.258183	0.240330
59.7993	19.0297	4.20636	2.14458	0.931358	0.966635	0.263608	0.249638
60.3205	19.0318	4.21774	2.16193	0.981595	0.969338	0.265779	0.262855
60.3700	19.1309	4.28801	2.17406	0.983528	0.981325	0.267724	0.265163
60.5046	19.2442	4.32179	2.20389	0.990748	0.998918	0.268560	0.267943
60.7523	19.3222	4.40951	2.21321	1.00385	1.02441	0.286185	0.277688
60.7923	19.4528	4.45023	2.21948	1.01754	1.02993	0.296741	0.293359
60.9737	19.6283	4.59329	2.27878	1.02162	1.04901	0.297775	0.306812
61.1269	19.6616	4.68681	2.28986	1.03389	1.05310	0.303585	0.309173
61.3211	19.7618	4.71736	2.30828	1.05638	1.08746	0.306396	0.328291
61.5540	20.0513	4.76979	2.36472	1.05738	1.09147	0.312241	0.362296
61.9556	20.0608	4.77154	2.50606	1.08479	1.10382	0.314086	0.364937
62.1315	20.1041	4.80334	2.50801	1.09343	1.11144	0.320725	0.394952
62.4223	20.1273	4.96781	2.52776	1.11548	1.11654	0.321034	0.397860
62.4975	20.1318	5.02231	2.56844	1.15281	1.15238	0.324664	0.428310
62.8218	20.3795	5.09761	2.63705	1.19403	1.18075	0.327135	0.449233
63.0643	20.4454	5.13994	2.73684	1.19940	1.19889	0.331056	0.452254
63.0989	20.4903	5.20571	2.75845	1.20806	1.21696	0.341912	0.457155
63.0997	20.5735	5.21791	2.76042	1.22161	1.26079	0.344177	0.466884
63.1257	20.7190	5.21912	2.79785	1.26676	1.27680	0.349538	0.473546
63.4060	20.7872	5.33642	2.86137	1.28188	1.28796	0.356381	0.549533
63.4686	20.8344	5.36956	2.92153	1.30656	1.30156	0.370518	0.555138
63.4724	20.9347	5.44547	2.98745	1.36106	1.33389	0.375000	0.561766
63.8065	21.1723	5.51781	2.99199	1.40637	1.34821	0.379155	0.593589
63.8217	21.3813	5.63852	3.01014	1.47431	1.37047	0.387592	0.602014
63.9691	21.3892	5.77289	3.01200	1.53354	1.38086	0.394699	0.604487
64.0441	21.4361	5.84637	3.04494	1.53734	1.38315	0.405647	0.624552
64.3342	21.5002	5.89000	3.09661	1.57239	1.39374	0.411382	0.654737
64.4179	21.5182	6.06558	3.13263	1.58365	1.43172	0.416441	0.671114
64.7153	21.5795	6.11624	3.14054	1.63338	1.47247	0.451081	0.685401
64.7573	21.6587	6.40879	3.14704	1.64075	1.47696	0.453361	0.685779
64.9332	22.0023	6.43262	3.15448	1.68618	1.54708	0.463423	0.694970
64.9424	22.0633	6.43746	3.23400	1.69157	1.56175	0.464677	0.703318
65.0609	22.1482	6.46792	3.29585	1.85089	1.56757	0.466471	0.720680
65.2266	22.3687	6.55751	3.30005	1.86858	1.59328	0.470896	0.742388
65.3611	22.5359	6.60016	3.33338	1.92694	1.60980	0.492403	0.754197
65.4581	22.5710	6.66743	3.40414	1.93265	1.61921	0.557848	0.761928
65.5184	22.7572	6.69689	3.40644	1.94161	1.63329	0.563277	0.762735
65.6210	22.8079	6.71789	3.50664	1.99511	1.64248	0.564160	0.769311
65.6866	22.9524	6.75383	3.50779	2.02526	1.65498	0.621309	0.782846

Table B.12 (cont.)

65.7025	23.1815	6.79345	3.52936	2.03560	1.69321	0.628664	0.797320
65.9914	23.2261	6.82077	3.55656	2.04866	1.71574	0.628995	0.830764
66.1429	23.3580	6.86346	3.56108	2.06539	1.75709	0.658653	0.841823
66.2313	23.3683	6.99992	3.57631	2.15401	1.81332	0.663987	0.868288
66.5071	23.4389	7.02641	3.60194	2.18746	1.84052	0.690657	0.927327
66.5589	23.4447	7.04190	3.61013	2.19323	1.91510	0.694165	0.955103
66.8286	23.6404	7.05491	3.63936	2.19527	1.93606	0.721342	0.963718
66.8508	23.6482	7.13327	3.64296	2.26765	2.04622	0.729064	0.992143
66.9705	23.6598	7.13974	3.64771	2.47045	2.05286	0.731687	1.02284
67.2894	23.6840	7.37254	3.66772	2.49705	2.09541	0.770872	1.06058
67.4299	23.7470	7.43063	3.77805	2.54093	2.25030	0.778084	1.07243
67.6922	23.9049	7.43250	3.81656	2.56447	2.31742	0.792471	1.08420
68.0385	23.9458	7.45878	3.82711	2.57831	2.32745	0.827652	1.10539
68.1680	24.0993	7.57235	3.92331	2.60903	2.33252	0.832929	1.12527
68.2961	24.3020	7.67779	3.96122	2.63238	2.34502	0.836319	1.15239
68.3528	24.3536	7.70315	3.97643	2.63570	2.35154	0.862770	1.18139
68.3914	24.7881	7.71181	4.01648	2.77225	2.37841	0.878977	1.25655
68.4374	24.8809	7.74150	4.10381	2.77732	2.46910	0.892099	1.27243
68.7219	25.1583	7.81750	4.13309	2.79645	2.53679	0.893653	1.27878
68.7238	25.1795	7.87461	4.21219	2.79824	2.54646	0.899753	1.30284
68.8437	25.2740	7.91272	4.21739	2.80281	2.64075	0.901565	1.30661
68.9303	25.4017	7.91949	4.30020	2.81038	2.68539	0.919337	1.32788
68.9781	25.7468	7.94930	4.35662	2.83088	2.73771	0.932843	1.33182
69.3508	25.7703	7.97691	4.64047	2.85144	2.74777	0.942193	1.33304
69.3859	25.8122	8.06780	4.65420	2.88759	2.78184	0.977574	1.35181
69.4769	25.9702	8.08335	4.71958	2.89063	2.83879	1.00817	1.38295
69.5247	26.0489	8.08351	4.82355	2.94749	2.87938	1.01467	1.38319
69.6196	26.4323	8.10676	4.89401	3.05237	2.92571	1.02528	1.43648
69.6273	26.7803	8.12816	4.92685	3.10740	2.99339	1.06087	1.44632
69.7967	26.8141	8.17817	5.00291	3.14601	3.03884	1.07930	1.47445
70.0414	26.9150	8.54299	5.06706	3.18148	3.03955	1.09441	1.50184
70.1054	26.9657	8.66728	5.11316	3.18728	3.15519	1.11058	1.57461
70.1207	27.2802	8.87803	5.14953	3.19469	3.15718	1.12436	1.59668
70.2971	27.2859	8.94200	5.17549	3.23120	3.18414	1.12882	1.61825
70.3628	27.3182	8.94340	5.34465	3.23984	3.19965	1.18652	1.65114
70.3860	27.3245	8.95004	5.49237	3.25488	3.24094	1.19131	1.70207
70.3899	27.4478	9.03964	5.58080	3.26081	3.29620	1.19395	1.71582
70.4276	27.4708	9.05676	5.58313	3.29048	3.31472	1.20419	1.73692
70.5615	27.5001	9.05923	5.74930	3.30521	3.32040	1.20586	1.81029
70.6267	27.5027	9.14470	5.76304	3.32308	3.37219	1.22250	1.82396
70.6499	27.6191	9.14655	5.78674	3.33667	3.43955	1.23012	1.83258
70.6844	27.8625	9.19569	5.94222	3.35035	3.44237	1.24840	1.83525
70.7648	27.8661	9.24869	5.94425	3.39547	3.44651	1.26326	1.84330
71.0606	27.9311	9.26007	5.94736	3.44344	3.45915	1.27255	1.86448
71.0689	28.2870	9.49478	5.97979	3.46958	3.49190	1.27580	1.95302
71.1774	28.4183	9.50906	5.98695	3.53001	3.49195	1.34193	1.97461
71.2055	28.7470	9.52282	6.00011	3.56093	3.50443	1.41090	2.04541
71.2201	28.7835	9.56610	6.03003	3.63074	3.56738	1.44762	2.07214

Table B.12 (cont.)

71.3132	28.7946	9.61136	6.06082	3.68509	3.59131	1.46000	2.09614
71.3991	29.0168	10.0444	6.28891	3.77126	3.59436	1.46099	2.11164
71.4179	29.1830	10.0744	6.30952	3.77374	3.61404	1.46663	2.12101
71.6171	29.3450	10.0861	6.32858	3.77491	3.67944	1.46752	2.14964
71.6788	29.4880	10.0975	6.35816	3.80665	3.76117	1.47153	2.15609
71.6870	29.5411	10.1288	6.40631	3.92205	3.82874	1.57246	2.16952
71.7416	29.7178	10.1743	6.43911	3.95393	3.83468	1.61653	2.18537
72.0391	30.0146	10.2858	6.52738	3.97971	3.91247	1.63922	2.23142
72.0393	30.0199	10.4715	6.61489	3.98073	4.05932	1.68685	2.24298
72.1475	30.0990	10.5463	6.62138	3.99378	4.06238	1.71492	2.28984
72.1967	30.2324	10.6396	6.66952	4.06283	4.12539	1.71547	2.31085
72.2577	30.3529	10.6808	6.80118	4.10583	4.13157	1.74366	2.32698
72.3735	30.3981	10.6952	6.80476	4.11019	4.13636	1.74384	2.33216
72.5176	30.4774	10.7319	6.82157	4.17284	4.16391	1.75918	2.34994
72.5664	30.6013	10.8981	6.88767	4.22502	4.16739	1.87552	2.35158
72.8219	30.7971	11.1141	6.94950	4.45553	4.17705	1.88597	2.37857
73.0362	30.9934	11.5120	7.06560	4.45711	4.22823	1.90279	2.41167
73.2360	31.1791	11.6071	7.09752	4.50640	4.43640	1.93258	2.41289
73.2641	31.2112	11.6099	7.12912	4.51730	4.45374	1.94547	2.46534
73.3328	31.2986	11.6383	7.14917	4.54712	4.53459	1.94761	2.47839
73.3841	31.4569	11.6998	7.18588	4.65204	4.63135	1.94909	2.50159
73.7065	31.5287	12.0164	7.24747	4.71316	4.64135	1.98806	2.51013
73.8321	31.6036	12.0662	7.27585	4.72136	4.64392	2.00214	2.55297
74.1049	31.6308	12.0831	7.29285	4.73075	4.66491	2.00716	2.58039
74.1514	31.6947	12.2287	7.38866	4.76629	4.67968	2.05472	2.68099
74.1970	31.7941	12.2396	7.43169	4.76854	4.68038	2.11381	2.76838
74.5311	31.8656	12.3277	7.43177	4.82242	4.71707	2.12412	2.78523
74.7666	31.9174	12.4827	7.45956	4.92787	4.71754	2.25880	2.81031
74.8418	31.9258	12.5195	7.48976	4.95644	4.74827	2.27512	2.82946
74.8978	32.0370	12.6660	7.59301	5.05658	4.80989	2.31195	2.98812
75.0490	32.0440	12.6836	7.60139	5.08149	4.84161	2.34052	3.05950
75.0597	32.1686	12.6942	7.68235	5.19099	4.85147	2.37604	3.07085
75.2880	32.1706	12.7757	7.68925	5.24560	4.88603	2.43129	3.11742
75.4504	32.3091	12.9792	7.72224	5.32978	4.91333	2.43379	3.11888
75.5911	32.3306	13.0903	7.79398	5.41575	4.99912	2.44891	3.19684
75.7700	32.6540	13.3289	7.88962	5.45480	5.08602	2.48529	3.20567
75.8075	32.6794	13.3834	7.90882	5.47394	5.13660	2.51739	3.21150
76.4715	32.7069	13.4340	8.02694	5.51382	5.22501	2.52016	3.25181
76.5542	32.7874	13.5702	8.13396	5.60541	5.23092	2.60513	3.25514
76.6133	32.8342	13.5926	8.13546	5.65417	5.24691	2.62068	3.31759
76.7081	33.1182	13.7948	8.15220	5.86294	5.24884	2.62468	3.40504
76.7760	33.1712	13.8570	8.19950	5.89695	5.32643	2.70041	3.41430
76.8393	33.5381	13.9423	8.23623	6.00389	5.40446	2.71168	3.43774
76.8910	33.5442	14.0033	8.25818	6.09601	5.40599	2.75834	3.45889
76.9595	33.6435	14.0605	8.37560	6.15021	5.50087	2.78623	3.47566
77.1093	33.6777	14.2878	8.41122	6.19945	5.58541	2.79801	3.48151
77.3571	33.8072	14.3043	8.51130	6.20407	5.70815	2.92264	3.62727
77.4220	33.9023	14.3727	8.51716	6.20848	5.71114	2.95012	3.70036

Table B.12 (cont.)

77.5391	33.9415	14.4120	8.53443	6.21574	5.73807	3.02729	3.90082
77.5429	34.0000	14.4310	8.71231	6.37146	5.76437	3.07878	4.13050
77.5585	34.9169	14.4315	8.73038	6.44291	5.78975	3.10872	4.14012
77.5677	34.9641	14.5155	8.84953	6.47619	5.79694	3.11639	4.18468
77.6582	35.1043	14.5667	8.90955	6.50950	5.84572	3.17123	4.21452
77.8129	35.1559	14.6378	8.94953	6.54777	5.88561	3.17612	4.24962
77.8293	35.2343	14.6904	8.97594	6.59295	6.11922	3.18274	4.34205
78.0061	35.4175	14.7189	9.01328	6.62885	6.14737	3.22183	4.38986
78.2782	35.5786	14.7583	9.05104	6.63650	6.18992	3.22211	4.52042
78.3480	35.6579	14.7611	9.07666	6.67723	6.19604	3.23462	4.54021
78.5841	35.7464	14.7927	9.13200	6.72724	6.25466	3.26757	4.55402
78.6607	36.4582	14.9055	9.21574	6.76420	6.31209	3.32691	4.58060
78.8863	36.4742	14.9199	9.24189	6.77380	6.32843	3.36079	4.64958
79.1639	36.5497	14.9348	9.24253	6.79128	6.45524	3.36411	4.71456
79.6337	36.6203	15.0082	9.24869	6.79571	6.47575	3.37707	4.78916
79.7965	36.7244	15.2147	9.34723	6.81872	6.74487	3.43391	4.84004
79.9450	36.9212	15.2527	9.37562	6.89788	6.82364	3.57061	4.85031
79.9698	37.1365	15.2565	9.40089	6.98339	6.85073	3.57756	4.88160
79.9834	37.3561	15.3141	9.44147	7.10560	6.95715	3.59214	4.90428
80.3975	37.6117	15.3824	9.50116	7.10813	6.96320	3.60011	4.98573
80.4123	37.6390	15.4094	9.56460	7.14602	6.96499	3.63403	5.00526
80.7146	37.6781	15.6082	9.58406	7.15906	7.00216	3.64299	5.03555
80.7173	37.7073	15.6897	9.61128	7.21332	7.01771	3.72108	5.04667
80.7275	37.7591	15.8601	9.61368	7.21924	7.19668	3.73541	5.06540
80.7411	37.8417	15.9443	9.62941	7.29747	7.25018	3.88342	5.06565
80.7505	38.1273	16.0044	9.77485	7.32567	7.27704	3.88842	5.07131
80.8560	38.2540	16.0593	9.86876	7.48291	7.55517	3.88919	5.08468
81.0738	38.4526	16.0672	9.99342	7.49675	7.55576	3.89614	5.08994
81.1310	38.9647	16.0979	10.0320	7.53857	7.59962	3.94632	5.18417
81.3346	39.3593	16.1619	10.0571	7.59543	7.62846	3.99698	5.21516
81.5519	39.7188	16.1663	10.0967	7.61073	7.64456	4.04173	5.44777
81.5572	39.8355	16.3849	10.3484	7.62525	7.73708	4.05062	5.47126
81.6072	40.2170	16.4670	10.4252	7.66155	7.78260	4.06110	5.53659
81.6316	40.7355	16.5063	10.4327	7.75768	7.81501	4.07869	5.54752
81.8079	40.8241	16.5438	10.5738	7.84653	7.88455	4.08949	5.56248
81.8439	41.0249	16.6018	10.5804	7.88143	7.88897	4.12346	5.59752
82.0160	41.2120	16.6414	10.7280	7.89509	7.91501	4.15664	5.74118
82.3199	41.2607	16.6477	10.7727	7.92387	7.93874	4.22134	5.81684
82.4909	41.3563	16.6724	10.8012	8.01449	8.03622	4.24433	5.81852
82.5699	41.3769	16.8607	10.8096	8.01817	8.08154	4.26778	5.82835
82.7766	41.4702	17.0258	10.8445	8.13121	8.09847	4.30765	5.83811
82.9126	41.8976	17.3448	11.1168	8.28634	8.14829	4.33280	5.87323
82.9554	41.9711	17.3700	11.1970	8.42084	8.25790	4.54604	6.02355
83.0073	41.9789	17.3923	11.2206	8.71803	8.55839	4.58414	6.03028
83.0793	42.0523	17.4620	11.3153	8.76504	8.56600	4.63661	6.21250
83.1968	42.1721	17.8179	11.4056	8.90037	8.75591	4.69350	6.28691
83.2623	42.2504	17.9589	11.4313	9.07792	8.84589	4.92562	6.30970
83.3615	42.4089	17.9836	11.5082	9.08605	8.87308	4.92770	6.37008

Table B.12 (cont.)

83.8344	42.4179	18.0374	11.5118	9.39646	8.90170	4.94204	6.40847
83.9253	42.5342	18.3454	11.5160	9.44691	9.01570	4.95661	6.43110
84.0972	42.5426	18.5352	11.5594	9.53232	9.08534	4.98584	6.43794
84.2678	42.6025	18.6299	11.5932	9.62175	9.11264	5.04326	6.52077
84.4045	42.7020	18.8037	11.5962	9.64085	9.13881	5.08559	6.52140
84.7026	43.0165	18.9925	11.6271	9.64530	9.19846	5.17471	6.59835
84.7717	43.2039	19.0835	11.6806	9.79820	9.21162	5.20257	6.63326
85.0031	43.2076	19.1567	11.7083	9.94481	9.52712	5.23877	6.64234
85.1122	43.2589	19.2540	11.7970	9.98748	9.56593	5.26453	6.65190
85.3422	43.3012	19.5152	11.8495	10.0727	9.61213	5.36073	6.76702
85.3575	43.4776	19.5565	11.8513	10.1026	9.61305	5.42347	6.87927
85.3880	43.8016	20.2753	11.9551	10.1749	9.65853	5.50931	6.91762
85.4241	43.9706	20.2775	12.1093	10.2213	9.75758	5.55128	6.92882
85.5095	44.2158	20.3060	12.2377	10.2374	9.78822	5.56691	6.97231
85.5541	44.3609	20.3065	12.2523	10.2528	9.94922	5.79785	6.97440
85.6165	44.4993	20.3068	12.2666	10.6324	10.1335	5.88514	6.99477
85.6668	44.5510	20.5474	12.3068	10.7307	10.3398	5.92649	7.01677
85.7429	44.5552	20.5720	12.3879	10.8854	10.3933	5.92935	7.33081
85.7527	44.6513	20.8763	12.4696	10.9467	10.3991	5.93894	7.33103
85.8171	44.7259	20.9694	12.4803	10.9521	10.6381	6.05788	7.36483
86.3151	44.8215	21.1598	12.5668	11.0507	10.6724	6.11212	7.37269
86.3601	45.2620	21.2540	12.5922	11.0774	11.0575	6.17312	7.39000
86.4234	45.5890	21.6208	12.7667	11.1757	11.0904	6.24274	7.40782
86.5009	45.9864	21.6615	12.7958	11.2066	11.6487	6.27825	7.45458
86.5542	46.0258	21.7222	12.8668	11.2621	11.6972	6.29369	7.45504
86.7139	46.0813	21.7374	13.0195	11.3113	11.7218	6.30064	7.46461
86.7964	46.0822	22.2702	13.1802	11.3352	11.8819	6.30494	7.51496
86.8870	46.1026	22.3856	13.1997	11.3998	12.0382	6.41154	7.54630
86.9281	46.1705	22.5611	13.2106	11.4436	12.0403	6.41538	7.69901
87.0540	46.2790	22.5899	13.5035	11.5463	12.2705	6.54993	7.85603
87.1234	46.4581	22.9737	13.6164	11.6738	12.4128	6.55843	7.98103
87.2135	46.4781	23.2754	13.9641	11.6743	12.6049	6.56059	8.05517
87.2237	46.5519	23.2829	14.0648	11.8416	12.6616	6.60722	8.07804
87.3206	46.6739	23.3341	14.1854	12.0548	12.8989	6.61041	8.09746
87.3583	46.6846	23.5437	14.1984	12.1272	12.9074	6.75746	8.13895
87.3811	47.0734	23.6059	14.2282	12.3797	12.9622	6.77595	8.14922
87.5357	47.1312	23.6347	14.2469	12.4723	13.0130	6.78301	8.19340
87.5636	47.7758	23.7464	14.3238	12.5904	13.0782	6.81633	8.19650
87.5896	47.8423	23.9204	14.5375	12.7729	13.1792	6.84364	8.22531
87.6922	47.9765	24.0492	14.5530	13.1340	13.2156	6.91627	8.24578
87.7272	48.2171	24.1083	14.7044	13.1558	13.4691	7.08550	8.32402
87.8353	48.5542	24.4139	14.8194	13.1871	13.5204	7.11261	8.34728
88.1220	48.8276	24.6471	14.9095	13.3004	13.7287	7.12271	8.39568
88.3253	48.9407	24.7476	14.9672	13.3304	13.7647	7.19379	8.50877
88.3301	49.4725	25.0574	15.1471	13.4570	13.8269	7.25263	8.58500
88.3426	49.6625	25.1825	15.3125	13.4908	13.9575	7.25674	8.64823
88.3785	49.8882	25.3978	15.3146	13.4994	13.9642	7.34362	8.67060
88.3932	49.9456	26.4038	15.3524	13.6217	14.3321	7.45546	8.69991

Table B.12 (cont.)

88.4646	50.0928	26.4575	15.5384	13.6418	14.3411	7.45808	8.89678
88.6262	50.1223	26.4687	15.7089	13.7542	14.4020	7.62178	8.91126
88.7275	50.1847	26.9809	15.8275	13.7608	14.4650	7.65722	8.95309
88.7896	50.7860	27.0158	15.8508	13.9808	14.4711	7.73476	8.96513
89.1128	50.8809	27.1078	16.1368	14.0777	14.5199	7.77012	9.01659
89.1249	51.0672	27.2392	16.3254	14.1028	14.9371	7.79118	9.09089
89.1896	51.5193	27.3448	16.4503	14.1309	15.0888	7.84246	9.17870
89.2457	51.7093	27.6781	16.5219	14.2092	15.2289	7.91809	9.28337
89.3318	51.8137	27.7586	16.5688	14.2162	15.2803	7.94763	9.31563
89.6143	51.8363	27.7911	16.6098	14.2265	15.7238	8.02609	9.34456
89.6560	51.8946	28.2992	16.6917	14.3423	15.7839	8.08565	9.41892
89.8182	52.0610	28.3824	16.7360	14.5692	15.8685	8.14510	9.43182
89.9579	52.6477	28.6542	16.8883	14.6522	15.9664	8.21528	9.44187
89.9677	52.7950	28.8202	16.9852	14.7142	16.1920	8.45048	9.46929
89.9827	52.8111	29.1463	16.9898	14.8042	16.7104	8.47543	9.55567
90.0720	52.8559	30.0891	17.1826	14.8822	16.7732	8.51388	9.67838
90.3055	52.8741	30.2238	17.5680	15.5613	16.8809	8.53288	9.95696
90.3732	52.9977	30.2565	17.8112	15.5864	17.1524	8.54877	10.1041
90.4101	53.5757	30.2650	18.1810	15.5904	17.3672	8.70040	10.1591
90.5979	53.6603	30.4252	18.2823	15.6701	17.7138	8.72987	10.1654
90.6028	53.7383	30.5117	18.4919	15.7763	18.0704	8.77174	10.1876
90.6560	53.7554	30.6524	18.5473	15.8162	18.1424	8.82154	10.2002
90.6916	53.8323	30.7921	18.5603	15.9713	18.2685	8.86780	10.2394
90.7666	53.8459	30.9767	18.6002	15.9892	18.3602	8.92651	10.3242
90.8278	54.1477	31.2421	18.6721	16.2346	18.6796	8.97908	10.3873
90.8785	54.1926	31.6934	18.7437	16.3309	18.8548	9.08494	10.7994
90.8853	54.2700	31.8082	18.8483	16.4064	18.9401	9.12720	10.8615
90.9827	54.2865	31.9226	19.1804	16.4951	19.0052	9.17575	11.0008
91.0625	54.3622	32.0873	19.3121	16.5845	19.1199	9.35270	11.0396
91.0824	54.3794	32.3047	19.3542	17.0185	19.1954	9.50546	11.0750
91.1221	54.5529	32.6399	19.5117	17.0400	19.2623	9.51554	11.2732
91.1814	54.6999	32.7526	19.9953	17.2090	19.3499	9.52155	11.5745
91.2282	54.7130	32.7581	20.0259	17.3720	19.3814	9.55797	12.0842
91.2949	55.0696	33.3348	20.0730	17.6012	19.4936	9.66794	12.1602
91.3713	55.2722	33.6230	20.3911	17.6370	19.6755	9.74422	12.1617
91.3913	55.5024	33.7607	20.8609	17.7905	19.9037	9.93224	12.2181
91.4670	55.6683	33.8992	21.0635	18.0868	19.9711	9.99371	12.3670
91.5539	55.8043	34.0412	21.2209	18.0887	20.2701	10.2140	12.3798
91.5829	56.0048	34.6124	21.6527	18.2320	20.3219	10.2387	12.9744
91.5840	56.0948	34.6651	21.7775	18.3980	20.3373	10.3728	12.9880
91.5870	56.4118	35.0311	22.0871	18.6162	20.3881	10.4860	13.0768
91.6399	56.4339	35.0521	22.7946	18.7640	20.5024	10.8760	13.0979
92.0412	56.5149	35.1798	22.8392	18.8107	20.5311	10.8818	13.1559
92.0801	56.5153	35.2729	23.1607	19.0487	20.6170	11.0339	13.1686
92.1478	56.6064	35.3610	23.2899	19.2615	20.7341	11.1282	13.2390
92.1949	56.6319	35.4376	23.4304	19.3824	21.0714	11.3936	13.6355
92.4050	56.8217	35.6287	23.4677	19.6114	21.9341	11.5446	13.7296
92.4060	56.8531	36.0987	23.4921	20.1595	21.9744	11.6108	14.3674

Table B.12 (cont.)

92.4174	56.9840	36.1996	23.6637	20.5315	22.0588	11.6846	14.4284
92.5664	57.0196	36.4364	24.0112	21.0909	22.3751	11.6962	14.5062
92.6670	57.2502	36.4840	24.2987	21.4911	22.8278	11.8001	14.6293
92.8592	57.4706	36.5934	24.3121	21.7697	23.2626	11.8693	14.7679
92.8913	57.6337	36.9442	24.3508	21.7979	23.4575	11.9265	14.8973
92.9894	58.0639	37.4812	24.7328	21.8736	23.6603	11.9499	15.0031
93.0388	58.5568	37.5831	25.1243	21.9058	24.2925	11.9654	15.2623
93.0525	58.9347	37.6797	25.4616	22.4932	24.5417	12.0548	15.2710
93.1148	58.9381	37.6921	25.4805	23.1143	24.6808	12.0696	15.4462
93.1181	59.0274	37.7695	25.5268	23.1956	24.7109	12.3112	15.7689
93.1336	59.0505	38.0837	26.0455	23.7175	25.6584	12.3141	15.7824
93.1780	59.0526	38.0975	26.6551	23.7326	26.1928	12.3240	15.9569
93.4576	59.1752	38.1103	26.8286	24.8931	26.5759	12.4070	16.0223
93.5937	59.2188	38.2088	26.9238	25.3260	27.1579	12.5486	16.2723
93.6689	59.2647	38.7386	27.1515	25.5152	27.2846	12.5562	16.3359
93.7822	60.0560	38.7487	27.1586	25.5926	27.8812	12.8768	16.3411
93.8226	60.4013	39.0066	27.5241	25.8424	27.9170	13.0485	16.6312
93.8505	60.6005	39.4489	27.9996	26.0347	27.9428	13.2188	16.8105
93.9652	60.9725	39.5236	28.3389	26.0425	27.9525	13.2671	16.8361
94.1161	61.2454	39.5340	28.6342	26.0464	28.0639	13.4130	16.8718
94.2102	61.4663	39.6901	28.6659	26.1764	29.0412	13.4372	17.2513
94.2555	61.5275	39.9489	29.4447	26.4986	29.0563	13.6595	17.3728
94.4120	62.1939	39.9902	29.4743	26.6398	29.4779	13.8167	17.4132
94.4161	62.2064	40.2564	29.7810	26.8815	30.0043	13.9128	17.6526
94.5509	62.3858	40.3157	30.2795	26.8944	30.8261	13.9187	18.1443
94.6022	63.2031	40.8999	30.8437	27.4418	31.1917	13.9218	18.3197
94.6819	63.2103	41.6529	31.1961	27.4900	31.2196	14.0624	18.3947
94.8491	63.3221	42.3339	32.5239	27.6988	31.3950	14.1389	18.5736
94.9303	63.9167	42.3784	32.6771	28.5001	31.5048	14.7864	18.7335
95.0954	63.9948	42.5639	32.8394	28.6043	33.0798	14.8061	18.8748
95.0958	64.1956	42.5970	32.9798	28.7284	34.6044	14.8992	18.9863
95.1474	64.6157	43.1611	33.1608	29.4892	34.6524	15.0706	19.9859
95.1891	65.0125	43.1710	33.3800	29.8150	35.4833	15.2872	20.1029
95.1903	65.5226	44.5119	34.3237	30.1254	36.5130	15.4254	20.1554
95.2306	65.6101	44.6066	34.6137	30.5786	36.7456	15.7235	20.6106
95.2635	65.6517	44.6608	35.1690	30.9518	36.7470	15.8497	20.6302
95.3727	66.7028	44.6687	35.2742	31.2739	37.1975	15.9130	20.8099
95.3936	66.8878	44.9016	35.7432	31.5032	38.9675	15.9547	21.7181
95.4192	67.3073	45.6319	36.1803	31.6711	38.9982	16.0441	22.2025
95.4267	67.4064	45.7139	36.3498	31.9256	40.2313	16.5328	22.6343
95.5990	67.4487	47.1549	36.7061	32.1833	40.5336	17.1124	22.7387
95.9177	68.1490	47.3060	37.9631	32.2745	41.3417	17.1635	22.9284
95.9595	68.7206	48.5792	38.2435	32.3230	41.4793	17.2615	22.9944
96.0831	69.3694	49.5377	38.3095	32.9679	41.6796	17.3310	23.2171
96.2985	69.4611	50.0400	38.6104	33.8518	42.1696	17.8293	23.9857
96.4272	69.6577	50.7184	38.9140	34.0136	42.8716	18.1931	24.0482
96.4598	70.7010	50.8017	40.3435	37.2645	44.2631	18.5196	24.3288
96.6312	71.7392	50.9967	41.1738	37.5944	44.6606	18.7314	24.3504

Table B.12 (cont.)

96.6565	73.2114	51.5804	41.2064	38.3258	45.0871	19.1605	24.3629
96.6823	73.3733	51.5966	41.7577	39.4997	45.5492	19.3914	24.9799
96.6940	73.9642	52.3220	42.4194	40.1541	45.9649	19.4952	25.5567
96.7013	74.4111	52.3708	43.2580	40.2337	47.0841	19.7897	26.6149
96.7382	76.0253	53.0300	43.4681	40.7611	47.6110	20.2243	26.9064
96.7443	76.4965	53.8556	43.8087	41.0338	48.5385	20.2961	27.2221
96.7680	76.6422	53.9361	44.0161	41.2683	48.8802	20.5583	27.5199
96.9609	76.9450	54.0627	44.0245	41.2943	50.8855	20.5639	27.9159
97.0058	78.0021	55.2751	47.2781	42.0423	52.0335	20.9302	28.2124
97.0484	78.2663	55.4787	47.9928	42.4769	53.7806	21.0898	28.2670
97.0699	79.1933	55.7309	49.3660	44.9404	53.7964	21.3365	28.7437
97.1416	80.9999	57.6883	49.5901	46.7511	54.9342	23.3688	29.9938
97.2406	83.5732	59.1545	51.6555	48.3681	55.8104	23.4569	30.5711
97.2997	83.9825	59.8507	52.8609	49.6305	56.8743	23.5640	31.1223
97.3396	84.9048	59.9358	53.2637	51.7543	57.7718	23.8244	35.4779
97.3706	85.7181	63.8634	54.8011	52.6653	58.3419	24.1233	35.9228
97.4841	85.9800	63.9154	55.7784	57.2822	60.6997	25.0361	35.9629
98.2184	88.5234	63.9687	59.4800	57.3900	64.1854	25.2705	41.3184
98.2289	88.6223	65.2724	60.3868	62.6293	65.1458	25.4974	44.8725
98.3698	90.0824	68.4644	65.3950	70.4624	70.2915	26.4511	48.3054
98.5123	91.4080	71.3508	72.4701	72.4519	70.7517	28.3941	55.2521
99.6025	94.4101	71.6963	72.9969	74.0827	83.4989	32.0680	85.0258
99.7362	95.7551	82.1573	98.7108	82.8518	84.4493	38.1853	85.6504

Table B.13 Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with BAN without and with 1, 2, 3, 4, 5, 10 and 24 hour liquid-holding recovery.

LH_0h	LH_1h	LH_2h	LH_3h	LH_4h	LH_5h	LH_10h	LH_24h
0	0	0	0	0	0	0	0
12.6755	0.030294	0	0	0	0	0	0
13.1433	0.052469	0	0	0	0	0	0
13.8361	0.096888	0	0	0	0	0	0
15.3620	0.104203	0.000353	0	0	0	0	0
16.8094	0.280657	0.000856	0	0	0	0	0
21.2838	0.301803	0.001609	0	0	0	0	0
21.4020	0.302163	0.003614	0	0	0	0	0
21.6680	0.330327	0.005395	0	0	0	0	0
24.0887	0.396323	0.010054	0	0	0	0	0
26.3767	0.398536	0.013638	0	0	0	0	0
27.8837	0.414194	0.016461	0	0	0	0	0
28.3504	0.477131	0.020126	0	0	0	0	0
29.4202	0.770173	0.020908	0.000701	0	0	0	0
29.4329	0.958396	0.023219	0.001524	0	0	0	0
30.7759	1.13672	0.058996	0.001567	0.000580	0	0	0
30.8328	1.59375	0.085221	0.002941	0.000724	0	0	0
31.6004	1.67672	0.088283	0.003572	0.000746	0	0	0.000882
34.0879	1.75861	0.115688	0.004643	0.002560	0	0	0.001049
34.2388	2.30007	0.129389	0.004741	0.003785	0.000562	0	0.001548
34.6999	2.41509	0.176493	0.006442	0.003838	0.000941	0	0.001670
34.7197	2.70482	0.268269	0.007122	0.004509	0.001219	0	0.001762
35.6816	2.89567	0.285522	0.011158	0.006062	0.001386	0	0.002298
36.4486	2.97515	0.300573	0.011167	0.006233	0.001910	0	0.002326
36.4833	3.48139	0.308477	0.011714	0.006985	0.002010	0	0.002330
36.8695	3.65273	0.332107	0.013876	0.007033	0.002574	0	0.002443
36.9331	3.69603	0.433248	0.016228	0.010209	0.002927	0	0.002446
37.8696	4.27580	0.449636	0.017770	0.010412	0.004682	0	0.002454
38.4510	4.32260	0.516902	0.018949	0.010824	0.006814	0	0.002802
38.6938	4.47140	0.523727	0.023692	0.011594	0.006849	0	0.003174
39.3644	4.47359	0.550740	0.024537	0.012747	0.007897	0	0.003277
40.2817	4.49848	0.571097	0.025156	0.014612	0.008037	0	0.004606
40.6451	4.65857	0.573898	0.029887	0.015414	0.008285	0	0.005147
41.1327	5.06049	0.589674	0.029925	0.015966	0.008960	0	0.007206
41.4688	5.14045	0.715847	0.030293	0.016227	0.010924	0	0.010922
42.1075	5.79873	0.769335	0.032784	0.016948	0.011646	0	0.011039
42.3518	5.83080	0.803501	0.034457	0.019444	0.013360	0	0.018562
43.1701	6.18087	0.943190	0.036100	0.023528	0.013532	0.000692	0.020522
43.9433	6.68975	0.967834	0.037979	0.024502	0.013629	0.000713	0.020957
45.0486	7.43439	1.04741	0.045029	0.028292	0.017858	0.001953	0.023344
45.4388	7.76338	1.11991	0.055830	0.030235	0.020216	0.003333	0.026584
45.5118	7.86473	1.12644	0.060274	0.031514	0.020276	0.003526	0.027576
45.7089	8.12647	1.14557	0.060628	0.035539	0.021181	0.003771	0.028213
45.7156	8.17176	1.15515	0.065045	0.038389	0.026014	0.003910	0.029892
45.7416	8.79017	1.17112	0.069158	0.038416	0.026354	0.004342	0.030444

Table B.13 (cont.)

45.9631	8.80634	1.49655	0.075399	0.039832	0.027259	0.004405	0.031125
46.0673	8.92617	1.55120	0.077130	0.045446	0.028262	0.005033	0.032377
46.2877	9.01183	1.64322	0.093205	0.048544	0.029195	0.005054	0.036924
46.5084	9.66902	1.76086	0.093345	0.048588	0.034284	0.005226	0.039999
46.5226	9.74549	2.35836	0.107104	0.056807	0.037333	0.005370	0.044116
46.5531	9.80358	2.43524	0.109106	0.073146	0.037373	0.005455	0.044718
46.6785	10.1945	2.53637	0.154917	0.082565	0.039822	0.005716	0.045518
47.0897	10.3777	2.59587	0.232068	0.083359	0.048881	0.005830	0.051808
47.2829	10.4676	2.68115	0.233671	0.087361	0.050157	0.008040	0.057714
47.4257	10.7966	2.71437	0.237802	0.090408	0.053662	0.008188	0.060244
47.5881	11.3635	2.73686	0.252224	0.097670	0.065219	0.008616	0.064963
48.0498	11.5920	3.12306	0.264124	0.108588	0.071981	0.008641	0.068582
48.7758	11.8383	3.18622	0.269220	0.126716	0.078690	0.009130	0.070470
48.8196	12.2376	3.20613	0.275367	0.132285	0.079552	0.009277	0.074258
49.4362	12.3190	3.30272	0.278601	0.136756	0.098811	0.009933	0.076030
49.7158	12.7729	3.31946	0.288006	0.152990	0.101354	0.011557	0.077552
49.7794	13.0562	3.36712	0.288428	0.154305	0.101401	0.012246	0.083625
50.1800	13.1864	3.47594	0.289883	0.179485	0.108275	0.012656	0.089931
50.3468	13.4355	3.76691	0.291625	0.181423	0.108511	0.012896	0.107984
50.5849	13.9775	4.00117	0.327628	0.193404	0.113121	0.014160	0.108188
51.2404	13.9911	4.09071	0.332165	0.195876	0.120318	0.014169	0.112112
51.4143	14.0883	4.09616	0.340000	0.198341	0.124180	0.015804	0.114746
51.4146	14.4229	4.21517	0.363603	0.220028	0.134089	0.015900	0.115079
51.8349	14.8964	4.23869	0.423358	0.231851	0.141139	0.018003	0.120994
52.3663	14.9411	4.37647	0.447477	0.240876	0.141387	0.020096	0.131434
53.1416	15.1568	4.47095	0.476234	0.254683	0.147483	0.020588	0.134072
53.2265	15.3831	4.49958	0.496363	0.344498	0.156823	0.023650	0.141399
53.6774	15.4894	4.81425	0.550634	0.349505	0.165234	0.023986	0.151545
54.4773	15.6102	4.90888	0.563148	0.352529	0.172191	0.026862	0.157964
54.9262	15.9752	4.95294	0.566728	0.353017	0.185593	0.028012	0.161263
54.9547	16.1378	4.97813	0.598895	0.395170	0.190781	0.030164	0.171174
55.3400	16.3034	5.13628	0.614349	0.400333	0.196145	0.031383	0.173002
55.4223	16.5220	5.23140	0.631864	0.440967	0.209679	0.031459	0.177306
55.5476	16.6934	5.29433	0.656936	0.455871	0.211678	0.036161	0.179224
55.9334	16.7083	5.96107	0.658339	0.461883	0.221448	0.040606	0.184799
56.3432	17.1957	6.11386	0.706040	0.488943	0.226661	0.045137	0.191940
56.3497	17.3892	6.71610	0.800773	0.530779	0.230277	0.049108	0.192150
56.8392	17.7955	6.75124	0.828661	0.562908	0.239686	0.051235	0.196669
56.8443	18.0105	6.89180	0.841472	0.652567	0.253660	0.052014	0.204246
56.9369	18.1113	6.89185	0.934185	0.655096	0.267782	0.052339	0.204837
57.1326	18.3575	6.91544	0.941375	0.703839	0.285587	0.056226	0.205521
57.4618	18.6621	7.07073	0.985385	0.709077	0.287620	0.058642	0.238646
57.7621	19.0244	7.28107	1.08377	0.712038	0.300326	0.070467	0.277461
57.7743	19.3118	7.29301	1.14801	0.717020	0.309154	0.072725	0.296946
58.0191	19.3443	7.36231	1.33203	0.717332	0.319237	0.075897	0.318597
58.2070	19.5269	7.44803	1.38882	0.814815	0.328467	0.076861	0.323773
58.3817	19.8198	7.52516	1.41320	0.865374	0.331977	0.083199	0.348114
58.9774	19.8800	7.54273	1.44684	0.881829	0.515517	0.086093	0.354031

Table B.13 (cont.)

59.3811	19.9595	7.82263	1.53853	0.955402	0.520717	0.086116	0.367144
59.5741	20.0103	7.97983	1.54438	1.07566	0.530306	0.089192	0.369462
59.5832	20.1997	8.02332	1.63007	1.08364	0.568845	0.092058	0.373402
59.6618	20.2066	8.21262	1.67944	1.09258	0.599631	0.10163	0.381995
59.7809	20.2092	8.30869	1.72207	1.13563	0.609600	0.10408	0.397289
60.0819	20.4175	8.33298	1.79986	1.13694	0.609729	0.10637	0.403094
60.1230	20.4719	8.39341	1.80303	1.19116	0.624213	0.12241	0.404134
60.1863	20.5719	8.44907	1.84910	1.20493	0.684774	0.12606	0.414586
60.2602	21.0124	8.48130	1.85763	1.20597	0.697903	0.13366	0.431080
60.3833	21.7143	8.67375	2.09170	1.22433	0.699169	0.15851	0.465948
60.6018	21.8970	8.76314	2.14593	1.22761	0.705951	0.16938	0.466655
60.6231	21.9806	8.78146	2.18693	1.25140	0.720893	0.17217	0.491473
60.8220	21.9999	9.01488	2.26444	1.25543	0.722257	0.17594	0.500154
60.8439	22.3795	9.08437	2.28672	1.26014	0.750444	0.18467	0.523299
60.8932	22.5540	9.15726	2.33032	1.26824	0.787264	0.19310	0.532267
61.1124	22.7215	9.41133	2.41901	1.27187	0.814645	0.22711	0.543252
61.5631	22.9643	9.49489	2.57346	1.29465	0.919949	0.24805	0.563476
61.9420	23.2552	9.56099	2.63810	1.34427	0.937480	0.25434	0.568262
62.4554	23.7107	9.95868	2.64718	1.42468	0.978670	0.28970	0.573489
62.4906	23.9390	10.6979	2.68073	1.46647	0.989019	0.30357	0.583923
62.6765	24.1356	11.4307	2.76761	1.53334	0.997406	0.31074	0.618733
63.1857	24.3579	11.5438	2.77957	1.54924	1.04104	0.34225	0.627954
63.1968	24.4017	11.8201	2.86994	1.56181	1.04295	0.36361	0.678601
63.4637	24.6958	12.5335	2.97374	1.59413	1.07852	0.37285	0.690721
63.7894	25.2727	12.6431	3.01197	1.65599	1.15799	0.37930	0.709041
63.8813	25.3036	12.6683	3.02706	1.66367	1.16537	0.38056	0.734878
63.9761	26.0413	12.7055	3.13012	1.70489	1.21345	0.39206	0.764974
64.3372	26.1348	12.7545	3.16126	1.71077	1.26439	0.39508	0.778271
64.5272	26.2784	12.8693	3.17680	1.80452	1.27196	0.39546	0.791148
64.5681	26.3977	12.9398	3.19046	1.80881	1.30555	0.43218	0.817331
64.8473	26.7763	13.0071	3.29320	1.80905	1.32956	0.43624	0.844790
65.1493	26.8184	13.2105	3.36015	2.00357	1.40819	0.44606	0.847812
65.3746	26.9071	13.2397	3.36860	2.05455	1.46443	0.46287	0.867517
65.5114	26.9431	13.2530	3.40461	2.09016	1.47081	0.47428	0.935574
65.7382	27.2438	13.3667	3.42125	2.20046	1.57837	0.49710	1.04179
65.7601	27.3789	13.6602	3.42726	2.22900	1.58900	0.50435	1.05169
66.0929	27.4278	13.7243	4.20336	2.25367	1.74376	0.50724	1.05259
66.1410	27.4800	13.7640	4.24823	2.30785	1.75767	0.53091	1.13724
66.2443	27.9186	13.9232	4.33684	2.34148	1.76573	0.54568	1.30517
66.9850	28.1287	13.9666	4.53804	2.35764	1.79403	0.58159	1.32189
67.0139	28.4117	13.9804	4.90415	2.36921	1.88122	0.58528	1.38231
67.2065	28.5365	14.0548	4.97360	2.36982	1.88971	0.60855	1.47798
67.4103	28.6012	14.2370	5.26822	2.45141	1.92667	0.65820	1.50891
67.5858	28.8939	14.2788	5.30879	2.57020	2.02535	0.68477	1.51807
67.9091	28.9008	14.3502	5.31556	2.57204	2.05707	0.72961	1.59754
67.9547	28.9955	15.2903	5.34834	2.66969	2.08626	0.73859	1.60567
67.9731	29.2111	15.4650	5.36138	2.71124	2.09375	0.74268	1.61256
68.2806	29.2366	15.7347	5.61341	2.78595	2.23892	0.79041	1.66321

Table B.13 (cont.)

68.3382	29.4114	15.7986	5.85769	2.90054	2.24932	0.79279	1.67155
68.4942	29.4855	15.8259	5.85885	2.96063	2.26625	0.82011	1.70261
68.7205	29.5031	15.8324	5.86947	3.00719	2.35686	0.89718	1.75337
68.7932	29.6385	15.8602	5.89672	3.02691	2.36165	0.90525	1.84502
68.8399	29.8050	15.9236	6.03539	3.14372	2.46043	0.91026	1.84614
68.9023	30.1875	15.9776	6.05637	3.24366	2.46788	0.92551	1.88809
68.9717	30.1952	16.0452	6.09494	3.31566	2.48325	0.95804	1.89890
69.0038	30.3271	16.0638	6.16361	3.39609	2.52318	0.98081	1.89963
69.0126	30.3720	16.4741	6.30502	3.51621	2.61698	1.00412	1.99559
69.0900	30.5178	16.5071	6.32127	3.60333	2.63331	1.01720	1.99781
69.3794	30.8716	16.5253	6.33588	3.72525	2.68013	1.03086	2.03145
69.3994	30.9054	17.1478	6.67572	3.74568	2.69572	1.06015	2.04654
69.4043	31.0865	17.3716	6.71386	3.85244	2.72538	1.06563	2.06037
69.5590	31.3637	17.4211	6.81899	3.94490	2.77833	1.06765	2.07289
69.8440	31.6188	17.6590	6.86677	4.06375	2.82937	1.09285	2.11855
69.8654	32.2021	18.0822	6.89097	4.11633	2.95161	1.10307	2.15726
69.9767	32.2402	18.4268	6.95535	4.26928	2.96613	1.12329	2.16797
70.0341	32.6182	18.4359	6.96605	4.30948	2.98929	1.13416	2.17234
70.1904	33.4675	19.1574	7.20213	4.57854	3.01683	1.14967	2.25671
70.3697	33.7551	19.9268	7.39571	4.63804	3.04183	1.16075	2.25881
70.4913	33.8480	19.9725	7.39595	4.69548	3.05388	1.18956	2.25991
70.5170	33.8722	20.2852	7.41129	4.70851	3.13514	1.21584	2.27450
70.5316	34.4731	20.4149	7.43728	4.76640	3.20252	1.22426	2.31679
70.7045	34.6413	20.5086	7.52329	4.83654	3.23076	1.25862	2.33590
70.9442	34.6484	20.6392	7.63959	4.94983	3.23562	1.30753	2.34143
70.9996	34.6524	20.6895	7.82746	5.00574	3.26187	1.37734	2.45530
71.0571	34.8889	20.7866	7.90364	5.06108	3.26601	1.38110	2.49024
71.1035	35.6384	20.8195	7.97046	5.06614	3.28801	1.38303	2.49050
71.1820	36.0357	21.0488	8.01513	5.12691	3.31730	1.43487	2.62163
71.5060	36.2897	21.3029	8.03225	5.23129	3.33926	1.46637	2.70095
71.5135	36.3954	21.9342	8.25354	5.39186	3.35639	1.47534	2.73462
71.8910	36.4262	22.3942	8.29773	5.45642	3.37693	1.50093	2.76165
71.9267	36.6911	22.4285	8.46336	5.57981	3.50256	1.65824	2.84792
72.0035	36.8039	22.5829	8.66188	5.58239	3.52518	1.66477	2.85960
72.0183	36.9515	22.8968	9.10408	5.61260	3.57167	1.74661	2.86910
72.0906	37.4537	23.0168	9.40650	5.70396	3.59495	1.76770	2.91078
72.1915	37.5304	23.0715	9.49109	5.72813	3.67354	1.76795	2.91602
72.2714	37.6294	23.0756	9.67693	5.73496	3.68306	1.88261	2.93747
72.4563	38.1503	23.2520	9.69817	5.79319	3.68940	1.97598	2.95097
72.5705	38.2311	23.2802	9.79247	5.92002	3.69597	1.98462	2.97963
72.9463	38.4480	23.6658	9.96095	5.92305	3.76699	2.08717	3.04712
73.0115	38.7394	23.9288	10.5160	6.23254	3.83559	2.14087	3.05354
73.0774	39.2300	24.1860	10.8131	6.25552	3.86198	2.18296	3.05837
73.1978	39.2420	24.5994	10.9214	6.32217	3.99927	2.19216	3.08041
73.2113	39.3606	24.8503	10.9655	6.35600	4.02877	2.21200	3.09880
73.2826	39.6277	24.9533	10.9884	6.41978	4.03257	2.27067	3.11831
73.7779	40.0561	25.5318	11.0500	6.46790	4.10197	2.36471	3.16278
73.7924	40.2332	25.6042	11.1084	6.47249	4.13724	2.36488	3.17496

Table B.13 (cont.)

74.0873	40.4331	25.9916	11.1270	6.54167	4.14750	2.37458	3.18492
74.1179	40.9571	26.0577	11.1364	6.57911	4.14943	2.40317	3.20288
74.1355	41.0790	26.2076	11.1965	6.58947	4.21720	2.41677	3.20419
74.1578	41.6086	26.2377	11.3626	6.66314	4.32755	2.45294	3.22341
74.5381	41.7021	26.2949	11.4709	6.70512	4.34056	2.47771	3.24989
74.5608	41.7058	26.4633	11.8781	6.79808	4.55337	2.48727	3.29697
74.8168	41.8080	26.9446	12.1292	6.80407	4.57387	2.51273	3.29913
74.9329	41.8302	27.0538	12.3513	6.83926	4.60236	2.69030	3.31519
75.0030	42.0033	27.2922	12.3610	6.85828	4.73276	2.73046	3.33522
75.0815	42.0486	27.3321	12.8559	7.00486	4.79950	2.77756	3.34173
75.2308	42.1542	27.8561	12.9079	7.15127	4.90163	2.78168	3.36645
75.8478	42.2621	28.0096	12.9549	7.26045	4.97135	2.79856	3.36980
75.9908	42.9986	28.0685	13.2292	7.35777	5.11244	2.90056	3.45986
76.1184	43.5195	28.1857	13.2413	7.36051	5.16324	3.00326	3.55677
76.2030	43.7423	28.2100	13.2532	7.36118	5.35425	3.07612	3.68506
76.9022	43.7642	28.4974	13.4116	7.54889	5.37578	3.24988	3.69806
76.9765	43.7871	28.8337	14.8172	7.58435	5.58644	3.26941	3.69880
77.3551	43.8144	28.8976	15.1676	7.72998	5.59685	3.31598	3.72538
77.3893	44.0257	29.0816	15.2174	7.81344	5.61747	3.34602	3.80374
77.5570	44.6094	29.1663	15.9269	7.88202	5.72964	3.36728	3.92927
77.6727	44.7065	29.4446	15.9366	7.92706	5.80700	3.41248	3.94837
77.7318	44.8264	29.4558	16.3959	7.94948	5.83881	3.42130	3.98759
78.1097	44.9451	29.6838	17.1763	8.01787	5.85582	3.44234	4.08627
78.1555	45.2011	29.6976	17.2668	8.05274	5.95300	3.45585	4.10676
78.2972	45.2736	29.7681	17.4943	8.05973	5.99075	3.53802	4.11235
78.3146	45.5036	30.0045	18.0005	8.08993	5.99376	3.61453	4.21258
78.5072	45.5135	30.2906	18.0569	8.15315	6.15824	3.62837	4.22883
78.7328	45.5716	30.9860	18.1711	8.18630	6.25183	3.71222	4.30723
78.7390	45.6798	31.5886	18.3444	8.21879	6.30782	3.84306	4.35262
78.8698	46.1871	32.1695	18.4039	8.29329	6.36652	3.86843	4.36752
78.9774	46.4529	32.3845	18.5005	8.37028	6.37957	3.88874	4.43006
79.4298	46.7912	32.6110	18.5648	8.41779	6.41036	4.00114	4.46351
79.7111	46.8284	32.7172	18.6195	8.66752	6.61396	4.11796	4.48246
79.8847	47.2503	32.7438	18.9396	8.73207	6.63603	4.24270	4.53497
79.8952	47.2676	32.7640	18.9899	8.95494	6.65236	4.25726	4.54956
79.9936	47.5142	33.2355	19.0580	9.01126	6.94379	4.25795	4.56198
80.0244	47.6560	33.2999	19.0923	9.06166	6.94800	4.25998	4.56271
80.0519	47.7905	33.4559	19.3453	9.18574	6.96078	4.28686	4.61833
80.0988	48.0181	33.7934	19.3759	9.36904	7.03820	4.35829	4.63450
80.1044	48.1337	33.9053	19.9170	9.44579	7.10519	4.39504	4.68435
80.1705	48.3707	34.0982	20.0770	9.47931	7.10535	4.40428	4.68933
80.6333	48.3907	34.3663	20.2305	9.52007	7.18901	4.53783	4.80078
80.7482	48.4042	34.4666	20.6035	9.57001	7.20328	4.53891	4.84292
80.8763	48.6902	34.9943	20.7550	9.81327	7.41725	4.55238	4.89872
80.9107	48.7877	35.1812	21.1527	10.2366	7.77897	4.57757	4.95681
81.5534	48.9504	35.3259	21.1936	10.4483	7.82462	4.58995	5.00488
81.6106	49.0266	35.8004	21.2818	10.6217	7.82594	4.59848	5.02447
81.6880	49.1121	35.8373	21.4960	10.8330	7.90271	4.67576	5.24501

Table B.13 (cont.)

81.8236	49.1491	35.8896	21.6273	10.8424	7.91071	4.69203	5.26545
81.9505	49.3964	36.2533	21.7597	10.8448	7.92531	4.80618	5.35591
82.0433	50.2250	36.5379	21.9329	10.9134	7.99173	5.20282	5.38017
82.3772	50.3965	36.7055	22.0806	10.9757	8.10951	5.23938	5.40885
82.5433	50.6354	37.6606	22.2828	11.0063	8.16977	5.29738	5.45098
82.5437	50.9301	37.8194	22.6029	11.2152	8.60721	5.30251	5.45139
83.0841	51.3607	37.8340	23.2847	11.2289	8.70386	5.33034	5.53956
83.3189	51.3722	37.8747	23.3784	11.2978	8.80795	5.37748	5.55809
83.5278	51.4769	38.0624	23.5832	11.4957	8.90314	5.38438	5.57867
83.5519	51.5391	38.1780	23.7150	11.5862	9.13661	5.46343	5.65248
83.6537	51.5994	38.4537	23.9415	11.6303	9.15047	5.47772	5.72329
83.7074	51.6138	38.6021	24.1436	11.7940	9.15283	5.50100	5.74781
83.7947	51.6369	38.6678	24.1657	11.8359	9.23336	5.52832	5.78772
83.8687	52.0321	38.9217	24.1949	12.1165	9.34219	5.61166	5.82690
83.9929	52.2917	39.3945	24.3172	12.1665	9.45391	5.71486	5.86985
84.0869	52.3548	39.9174	25.1524	12.2109	9.46968	5.80791	5.91972
84.1072	52.3675	40.3116	25.1667	12.5074	9.48370	5.85295	6.05213
84.1802	52.4686	40.3953	25.4435	12.6116	9.63120	5.88681	6.19763
84.4195	52.6142	40.9685	25.6187	12.6896	9.84778	5.93813	6.35284
84.5499	52.6974	41.2990	25.7461	12.7775	9.92286	6.04432	6.38793
84.6490	52.7780	41.7535	25.7927	13.0118	9.99766	6.04646	6.39297
84.6576	52.9048	41.9613	26.0829	13.0602	10.1109	6.07674	6.64861
84.7221	52.9951	42.1326	26.6746	13.3720	10.2873	6.14361	6.65721
84.9969	53.1357	42.2636	26.7632	13.5291	10.3069	6.23474	6.67054
85.0446	53.6591	43.2076	27.0094	13.6111	10.4053	6.27733	6.68460
85.2336	53.7191	43.2282	27.0395	13.6378	10.8334	6.33296	6.68648
85.2465	53.8117	43.2626	27.0460	13.9382	11.1792	6.33646	6.69088
85.2763	53.9755	43.3894	27.3914	14.7665	11.2903	6.40545	6.69839
85.3656	54.3379	43.5142	27.4191	15.1590	11.3031	6.44325	6.73954
85.5048	54.4214	43.5350	27.7009	15.2379	11.4145	6.48899	6.74689
85.5538	54.9866	43.6456	27.9458	15.2464	11.5028	6.62321	6.84667
85.6013	55.3129	43.6753	27.9618	15.8646	11.5151	6.67845	6.87704
85.6195	55.5444	43.6871	27.9754	15.9710	11.5472	6.80226	7.30044
85.8323	55.6837	43.7614	28.0603	16.0635	11.7351	6.90120	7.33795
85.8450	55.7545	43.9361	28.2582	16.3861	11.7582	6.90151	7.34289
85.9238	56.2510	44.4422	28.2615	16.4029	11.7915	7.03063	7.35156
86.2454	56.5437	44.5219	28.4212	16.7470	11.8543	7.29374	7.45731
86.3435	57.5950	44.5515	28.5907	17.1085	11.8605	7.50186	7.45746
86.5818	57.9886	44.6391	28.7277	17.3946	11.8896	7.52446	7.47946
86.7425	58.2539	44.8710	28.9482	17.4803	11.9355	7.56324	7.53362
86.7575	58.3231	44.8759	30.0990	17.7056	12.0183	7.79398	7.53574
86.8013	58.4201	45.1405	30.1997	17.9529	12.2530	8.01365	7.55262
87.0035	59.0703	45.3290	30.2772	18.0271	12.3912	8.07155	7.62962
87.0657	59.2958	45.4105	31.4608	18.5605	12.4998	8.32422	7.64992
87.1173	59.4617	45.4458	31.6562	19.5553	12.5297	8.43886	7.83926
87.2571	59.7048	45.6126	31.6583	19.6235	12.5866	8.48467	7.84855
87.2892	60.0703	45.8435	32.1408	19.6715	12.6874	8.51028	7.94226
87.5381	60.1330	45.9100	32.4509	19.7150	12.7192	8.57052	7.99240

Table B.13 (cont.)

87.5506	60.1853	46.4809	32.5502	19.9698	12.7271	8.66309	8.00234
87.5992	60.9028	46.7021	32.6351	20.0174	12.7772	8.67897	8.06757
87.6538	61.1252	46.9767	32.9069	20.0232	12.9648	8.68461	8.12425
87.7137	61.1421	47.2068	33.4763	20.7615	12.9690	8.76279	8.25649
87.7816	61.4395	48.5811	33.4997	20.9475	13.4443	8.94310	8.30969
87.7879	61.6066	48.6046	33.5897	21.1236	13.6539	8.94665	8.55754
87.8924	61.7501	48.7211	33.5910	21.3314	14.0627	9.10713	8.64507
87.9011	62.2042	48.9704	34.3141	22.0649	14.0809	9.12704	8.64524
87.9590	62.2364	49.4314	34.5675	22.0662	14.2072	9.15651	8.83111
88.1533	62.4189	49.7580	35.1148	22.9898	14.2417	9.20225	8.84686
88.2920	62.5412	50.0753	35.9942	23.0453	14.2423	9.32346	8.91676
88.4616	62.5420	50.1793	36.2730	23.2441	14.7170	9.48045	8.95899
88.5689	62.8768	50.2046	36.3778	23.5744	15.1049	9.54142	9.11937
88.6699	62.9351	50.2770	36.4476	23.9410	15.2825	9.57920	9.45914
88.7854	63.1567	50.6988	36.5043	24.9737	15.2982	9.60255	9.64341
88.9433	63.4859	51.1349	37.1375	25.4837	15.4164	9.97583	9.68412
88.9460	63.5453	51.3903	37.5707	25.6599	15.5271	10.0681	9.77827
89.1168	63.5576	51.6449	37.6284	25.9519	15.6981	10.1057	9.82572
89.1240	63.5699	51.9925	38.0155	25.9787	15.8154	10.1088	9.83237
89.2848	63.5701	52.0584	38.1044	26.4018	16.5922	10.3885	9.83370
89.3569	63.6797	52.3318	38.2377	26.9865	16.6878	10.3933	10.0123
89.5533	63.7181	52.6324	39.1078	27.0255	16.9147	10.4349	10.3628
89.5610	63.9881	52.6373	39.7307	27.0437	17.1125	10.5337	10.4705
89.6020	64.0348	53.1874	40.1572	27.2932	17.6098	10.5376	10.4998
89.7087	64.1258	53.5639	40.5076	27.5786	17.8580	10.7030	10.6467
89.8540	64.1292	53.9387	41.0977	27.6532	17.9461	10.8035	10.6829
90.0028	64.4233	54.2919	42.1355	28.3013	18.0652	10.9564	10.7577
90.0429	64.9186	54.3069	42.8384	28.3857	18.1400	11.0316	10.8673
90.0781	64.9201	54.5096	42.8524	29.2099	18.2767	11.4706	11.0142
90.2647	65.2762	54.5380	43.8255	29.3170	18.4389	11.6278	11.0306
90.3402	65.3902	54.7171	43.8713	30.2444	18.6177	11.8217	11.0551
90.4818	65.7442	54.7725	43.9032	30.5172	18.6812	11.9509	11.2155
90.5237	66.2842	54.9087	44.4878	30.6068	18.6903	12.0338	11.2725
90.5716	66.3256	55.2965	44.6467	30.6591	18.8398	12.0923	11.3785
90.7525	66.4650	55.6193	44.9931	31.6944	18.9880	12.1440	11.5331
90.8767	66.9527	55.7570	45.0078	32.1314	19.2067	12.1925	11.5423
90.9559	67.0863	55.7899	45.6365	32.1394	19.2909	12.3086	11.5604
90.9685	67.6008	55.9512	45.8607	33.7747	19.5020	12.8226	11.6866
91.2963	68.0773	56.4599	46.0259	33.8030	19.6460	12.9104	11.7015
91.3847	68.2316	57.7702	46.6155	34.2675	19.8501	13.0520	12.3212
91.4508	68.4463	58.1631	47.0391	34.3127	19.8574	13.0719	12.4245
91.6213	68.7234	58.4943	47.0760	34.9508	19.9137	13.1832	12.4563
91.6362	68.9807	58.5189	47.2376	34.9553	19.9891	13.2602	12.7816
91.6864	69.1975	58.5225	47.4119	35.6780	20.1626	13.3473	12.8566
91.8396	69.6955	58.6262	47.7339	36.1084	20.4307	13.6374	13.1228
92.2151	69.7235	58.7157	47.8023	36.7733	20.8539	13.6704	13.1609
92.3399	70.1486	58.9641	48.0974	37.6875	21.0298	13.7169	13.3110
92.4783	70.1545	59.0791	48.2314	38.8031	21.8974	13.7258	13.4163

Table B.13 (cont.)

92.5814	70.5753	59.1552	48.4626	39.3351	22.1654	13.7674	13.4822
92.7818	70.9780	59.2812	48.8039	39.6593	22.3996	13.7732	13.5470
92.7986	71.0266	59.4920	49.0019	40.0416	22.7775	13.8143	13.7775
92.8829	71.4621	59.8054	49.3546	40.0515	23.0710	13.9051	13.8532
93.0128	71.4732	59.8567	49.6692	40.8970	24.1254	14.0460	13.8763
93.0549	71.8420	60.0886	49.7002	42.4772	24.2597	14.1268	14.4225
93.5873	72.3361	61.0636	49.7120	42.7628	25.7706	14.1497	14.5701
93.7515	72.6707	61.2194	50.1528	42.7695	25.9125	14.1629	14.8777
93.9581	72.7110	61.3393	50.3388	43.2044	26.0749	14.2327	14.8990
94.1854	72.8713	61.4905	51.0189	43.9063	26.4321	14.5363	14.9415
94.3373	73.1245	62.1003	51.6634	44.8018	26.4557	14.7911	14.9537
94.4317	73.2077	62.1008	51.7483	45.4003	26.5060	15.3862	15.0708
94.4492	73.6456	62.2324	52.5605	45.4307	27.4574	15.8903	15.0875
94.4533	73.9246	62.5352	53.3100	45.9521	27.6083	15.9948	15.1446
94.7060	74.0730	62.8894	54.1626	46.7900	27.7519	16.5027	15.3379
94.7270	74.1270	64.0761	54.5077	47.3920	28.2647	16.7753	15.6338
94.8484	74.2449	64.4363	54.7674	48.1091	28.3303	16.7894	15.6702
94.8943	74.6251	65.3537	54.8658	49.0288	28.9354	16.8490	15.8445
94.9185	74.8580	65.5943	55.0796	49.0893	29.0189	16.8756	15.9246
95.0188	75.1719	65.6235	55.3401	52.2138	29.3705	16.8821	15.9966
95.2221	75.3155	66.8760	55.6647	52.5915	29.5592	17.0151	16.0527
95.2575	75.3751	67.0456	56.4857	52.7257	29.8734	17.1599	16.0801
95.6608	75.9549	67.4020	57.1642	53.4466	30.6741	17.7627	16.1207
95.7679	76.3730	67.6387	58.1154	53.5373	30.8800	18.9527	16.1997
95.8884	76.7600	67.6521	59.0421	56.6503	30.9537	19.1134	16.9656
96.0377	77.0602	68.0016	59.6216	57.2641	31.3690	19.2360	17.0493
96.0738	77.2245	68.0597	59.6985	57.2717	31.4547	19.2941	17.0672
96.1133	77.3295	68.8929	59.7233	57.4845	33.9851	19.8587	17.0894
96.1678	77.4798	69.1827	59.7933	57.6043	35.2821	20.3060	17.3801
96.2198	77.4800	70.3446	59.9068	57.9996	37.3296	20.3651	17.5737
96.2393	77.5560	70.9504	60.7148	58.8160	37.7135	21.1282	17.5857
96.6859	77.6012	71.0273	61.0554	58.8462	37.7589	21.4116	17.6974
97.1570	77.8224	71.3707	62.0547	59.1662	38.1379	22.0454	17.9148
97.2145	77.9868	71.6616	62.3996	59.5007	39.3438	22.8934	17.9795
97.3438	78.1880	72.1243	62.7160	59.7711	39.4586	23.1014	18.2016
97.3825	78.4247	72.6883	63.8172	61.4192	39.8115	23.1191	18.4412
97.4522	79.0273	73.2496	64.1460	62.4194	40.0365	25.2488	18.9001
97.5403	79.6146	73.9638	64.9014	63.4680	40.1459	25.3465	19.5627
97.5693	80.1844	74.0352	65.7185	66.6240	40.8888	25.3533	19.8197
97.7717	80.3583	74.1655	66.8184	66.6870	41.3229	25.8972	20.7091
97.9751	80.6121	74.2813	67.9576	67.6444	43.0367	26.9226	20.7277
98.0068	81.4150	75.0514	68.2047	67.7283	44.9132	28.1181	20.9504
98.1093	81.4192	75.6374	68.7102	67.7528	45.4386	28.3392	21.2489
98.2421	82.2090	76.1098	69.3501	67.9344	46.2013	29.6640	21.3929
98.3097	82.2598	76.6567	69.7836	68.0568	46.2859	30.0306	21.5135
98.5352	82.4033	77.2796	70.3059	68.3141	51.6373	30.3432	21.6327
98.5611	83.1302	77.5068	70.5319	68.4920	54.0725	30.4292	21.7895
98.6972	83.5088	77.6020	71.1950	71.8426	54.7669	31.7484	22.2208

Table B.13 (cont.)

98.7674	83.7000	78.0393	71.3417	73.7486	56.0282	33.1305	23.4938
98.9380	83.8155	78.2125	72.7212	74.8232	56.0687	33.5398	23.6788
99.0814	84.0011	78.5360	73.5333	75.3027	58.4727	34.4653	24.2220
99.2276	84.2012	79.2363	73.5872	76.8542	59.0805	35.2191	24.6054
99.3470	84.5908	79.2942	73.9363	76.9899	61.7230	35.7826	24.6807
99.3526	85.5561	82.9504	74.1686	79.0198	61.7746	37.5617	25.4492
99.3550	86.6946	82.9739	75.1955	79.5697	62.1362	38.4414	26.8021
99.3754	87.3003	83.1136	75.5555	80.0257	62.1833	40.1298	27.8530
99.4058	87.5308	84.3834	76.5204	80.4632	64.1072	42.6556	28.3945
99.5735	88.5143	85.4111	78.8907	81.0932	64.7758	42.7222	30.5140
99.5831	89.7295	85.9626	80.6545	81.1514	69.3383	45.7774	33.6721
99.6198	89.7595	86.8360	82.3114	84.4836	70.8502	50.0730	34.4898
99.6368	92.1350	86.8697	82.3370	84.5038	78.0797	58.3611	36.4979
99.7800	92.7515	86.9063	82.8157	85.0261	79.2471	58.6137	38.4766
99.9277	94.2612	93.5279	85.3328	86.1266	79.4082	69.2058	42.9457
99.9555	95.2679	93.8205	86.9426	86.5445	79.6864	72.4235	43.2171
100.0000	95.8026	94.3323	91.1442	95.7499	80.7127	72.8642	62.4487
100.0000	95.9860	97.0737	96.7816	97.3545	81.4152	74.1397	78.5475
100.0000	96.6843	98.1207	97.5905	97.7896	87.3975	76.9578	79.9890

Table B.14 Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from CHO cells treated with IAN without and with 1, 2, 3, 4, 5, 10 and 24 hour liquid-holding recovery.

LH_0h	LH_1h	LH_2h	LH_3h	LH_4h	LH_5h	LH_10h	LH_24h
1.72184	0	0	0	0	0	0	0
3.99319	0	0.001786	0	0	0	0	0
5.12593	0	0.007236	0	0	0	0	0
5.49612	0	0.012873	0	0	0	0	0
5.83861	0	0.013350	0	0	0	0	0
6.13382	0	0.070836	0	0	0	0	0
6.62325	0.001075	0.083221	0	0	0	0	0
6.90653	0.001913	0.222110	0	0.000783	0	0	0
9.02286	0.003892	0.291282	0.000290	0.001498	0	0	0
9.45433	0.006735	0.314562	0.005095	0.001832	0	0	0
10.5645	0.021456	0.399386	0.005246	0.004931	0	0	0
11.1126	0.028897	0.404258	0.007114	0.007073	0	0	0
11.8901	0.047178	0.410043	0.016886	0.007508	0.000810	0	0
11.9600	0.056643	0.472388	0.026916	0.007771	0.000831	0	0
13.6143	0.151036	0.549526	0.031748	0.007991	0.001892	0	0
13.9864	0.174958	0.557900	0.038594	0.010148	0.003133	0	0
14.0261	0.400716	0.676172	0.050804	0.017626	0.004220	0	0
14.2828	0.487478	0.750813	0.057119	0.018350	0.006818	0	0
14.4038	0.512611	0.814032	0.062572	0.019199	0.011357	0	0
16.7149	0.526954	0.888799	0.091778	0.019213	0.011803	0	0
17.4530	0.563144	1.04400	0.111331	0.026763	0.013338	0	0
17.5834	0.717309	1.13235	0.116886	0.027491	0.013930	0	0
17.7194	0.737133	1.29112	0.143717	0.032295	0.014387	0	0
17.9994	0.889371	1.31824	0.149158	0.033342	0.022834	0.000974	0
18.1989	0.996222	1.35223	0.157824	0.034123	0.037815	0.001400	0
19.4038	1.21112	1.55364	0.183048	0.034415	0.040822	0.001526	0
21.1739	1.23383	1.79311	0.184406	0.038183	0.041733	0.001562	0.000810
22.3809	1.27897	1.92684	0.243597	0.052181	0.062854	0.001902	0.001049
23.0680	1.38043	2.15188	0.252808	0.065775	0.068434	0.001943	0.001260
23.3288	1.40432	2.34010	0.267402	0.066332	0.069589	0.002362	0.001560
24.5901	1.52619	2.35232	0.281471	0.080588	0.071209	0.003097	0.001695
25.0046	1.52730	2.39498	0.310713	0.081410	0.073294	0.003453	0.001888
25.5721	1.58291	2.59158	0.316154	0.082219	0.075130	0.004662	0.002697
25.6368	1.66829	2.64876	0.367345	0.084421	0.082658	0.004668	0.004154
25.9498	1.95557	3.19455	0.390607	0.084954	0.085060	0.004806	0.004545
26.5786	2.21250	3.42960	0.426980	0.095906	0.086664	0.005646	0.005856
27.0796	2.25524	3.69938	0.441037	0.098647	0.086951	0.007379	0.007173
27.7880	2.40605	3.72755	0.477715	0.101015	0.087784	0.008077	0.007675
27.8759	2.46590	3.77177	0.478316	0.106312	0.103567	0.008337	0.007748
28.1671	2.51821	3.87378	0.490589	0.122866	0.109123	0.008721	0.009683
28.2595	2.60860	3.91608	0.522269	0.124824	0.110132	0.009492	0.010127
28.6592	2.62531	4.29670	0.536395	0.138176	0.115631	0.011629	0.010200
29.0453	2.66254	4.30031	0.570793	0.168877	0.118044	0.012042	0.010773
29.4376	2.71214	4.32354	0.591323	0.172701	0.131827	0.012517	0.011873
29.8052	2.72784	4.64108	0.631806	0.174115	0.138423	0.013613	0.011990

Table B.14 (cont.)

29.8087	2.78637	4.68497	0.647348	0.183042	0.143618	0.013872	0.014764
30.1207	2.80605	5.04157	0.694476	0.210009	0.150941	0.014262	0.015133
30.1273	3.12613	5.08002	0.702133	0.236076	0.159432	0.015254	0.017390
31.0259	3.12707	5.27920	0.721452	0.285938	0.173302	0.017477	0.024777
31.2040	3.15106	5.42961	0.754406	0.286379	0.187877	0.017725	0.025824
31.3918	3.18786	5.59203	0.821193	0.321244	0.194336	0.018823	0.026429
31.5257	3.28100	5.70246	0.868657	0.339210	0.195822	0.020372	0.026623
31.7572	3.30647	5.74332	0.883224	0.339499	0.200618	0.028160	0.037620
31.7613	3.50011	6.00871	0.923566	0.402767	0.202227	0.030663	0.041040
31.8104	3.51214	6.12809	0.932340	0.432135	0.211266	0.033866	0.048743
32.9744	3.56025	6.15863	0.949633	0.482633	0.217062	0.037045	0.048808
33.1960	3.82834	6.17264	0.954004	0.483570	0.245469	0.039495	0.054550
33.2216	3.95677	6.31929	1.04374	0.502913	0.246765	0.040650	0.057069
36.0350	4.39413	6.36152	1.09806	0.520322	0.254871	0.041647	0.069045
36.1574	4.85138	6.49235	1.11612	0.563490	0.280398	0.046884	0.072650
36.3893	5.12706	6.55980	1.16224	0.575742	0.284059	0.053702	0.076060
36.3941	5.27284	7.18902	1.25972	0.578919	0.294520	0.054308	0.076522
36.5467	5.29691	7.38552	1.26668	0.585215	0.309078	0.055670	0.080850
36.9896	5.46498	7.50922	1.31708	0.607725	0.355101	0.057455	0.086458
37.2867	5.51617	7.85316	1.48880	0.620426	0.355197	0.065676	0.088246
38.0915	5.61947	8.05400	1.49079	0.627010	0.378757	0.071908	0.088397
38.3961	5.67268	8.53562	1.49474	0.633389	0.463101	0.073474	0.089087
38.8283	5.75222	8.64816	1.49632	0.663159	0.498310	0.077804	0.089406
39.5948	6.12242	8.76998	1.62080	0.663239	0.509566	0.082376	0.097384
39.8920	6.17219	8.91651	1.68079	0.675752	0.513387	0.082612	0.104962
40.6865	6.36540	9.03064	1.74355	0.692961	0.532945	0.083445	0.109318
41.1445	6.64250	9.18279	1.76661	0.711123	0.567106	0.092290	0.114579
41.3721	7.59458	9.22590	1.83157	0.740291	0.577216	0.095686	0.117696
41.8886	7.69186	9.26581	1.83935	0.744240	0.600339	0.101412	0.124251
42.0813	7.86560	9.38334	1.90606	0.765931	0.692873	0.102705	0.129972
42.2297	8.08137	9.46667	1.93412	0.853901	0.697686	0.121996	0.134484
42.2411	8.15204	9.62290	2.09918	0.861738	0.754827	0.141256	0.145480
42.3897	8.42938	9.65845	2.12921	0.873466	0.768752	0.150933	0.146703
43.3265	8.60536	9.76137	2.24974	0.923483	0.832558	0.198300	0.147963
43.4535	8.75789	9.92946	2.28510	0.934293	0.898021	0.202651	0.157524
43.6774	8.93548	10.0053	2.42610	0.980439	0.931506	0.207469	0.199836
43.6962	9.17148	10.0472	2.45714	1.02281	0.945984	0.222015	0.208252
43.7044	9.86429	10.1139	2.53964	1.07069	0.973774	0.243861	0.213354
43.9268	10.1162	10.1367	2.54024	1.11986	1.01440	0.250913	0.231338
43.9341	10.1887	10.4671	2.56432	1.21203	1.03586	0.262603	0.238917
43.9391	10.2502	10.7517	2.64124	1.27386	1.05564	0.280120	0.246500
44.2388	10.3110	10.7837	2.68513	1.33059	1.05794	0.283338	0.249400
44.4151	10.3678	10.9917	2.70165	1.33613	1.07015	0.289035	0.274307
44.8727	10.4092	11.0857	2.71833	1.36127	1.07528	0.294709	0.279491
44.9579	10.7991	11.4344	2.72928	1.36600	1.07635	0.315453	0.287115
45.3865	10.9040	11.5993	2.73109	1.38005	1.09986	0.379547	0.300348
45.4707	11.0731	11.6139	2.76827	1.52696	1.10450	0.389494	0.306500
45.9568	11.0946	11.7080	2.83836	1.54812	1.13360	0.398297	0.311145

Table B.14 (cont.)

46.2769	11.5247	11.8439	2.88015	1.57027	1.13454	0.424434	0.314295
46.3146	11.6564	11.9074	2.91148	1.60537	1.14813	0.432402	0.349515
46.7179	11.6758	12.0454	2.98502	1.63868	1.18241	0.449041	0.390784
47.0575	12.0243	12.1574	3.08657	1.67694	1.21425	0.458633	0.391580
47.4027	12.3500	12.2873	3.18291	1.68773	1.31320	0.473177	0.417423
47.6869	12.5746	12.3611	3.20889	1.70407	1.33471	0.505377	0.433257
47.7306	12.6553	12.4236	3.30971	1.73620	1.37321	0.507767	0.436399
47.7764	12.8278	12.4538	3.34079	1.73924	1.38850	0.536780	0.450586
47.8412	12.9161	12.6787	3.39742	1.77605	1.43992	0.595485	0.458472
48.0724	12.9593	12.7458	3.41098	1.79333	1.45592	0.623140	0.471686
48.4671	13.4759	12.8943	3.43941	1.85813	1.55595	0.669516	0.492381
48.5576	13.5647	13.1941	3.44163	1.88379	1.55907	0.675950	0.515827
48.7738	13.6026	13.3441	3.45806	1.97673	1.65848	0.695293	0.580172
48.8869	13.6994	13.3690	3.46409	2.03822	1.74980	0.718820	0.581264
49.0950	13.7416	13.8251	3.48088	2.08430	1.79461	0.761216	0.584220
49.1759	13.8632	13.9003	3.49865	2.16959	1.81117	0.783771	0.615765
49.4096	13.9089	14.0506	3.52399	2.21133	1.84419	0.822022	0.635436
49.7543	14.0267	14.1413	3.56768	2.23072	1.88672	0.832013	0.638069
49.7682	14.4240	14.8754	3.61488	2.36238	2.03617	0.843369	0.651801
49.7926	14.5147	15.1136	3.62101	2.43413	2.20716	0.915905	0.697220
50.3722	14.5429	15.1869	3.79920	2.44090	2.22379	0.964565	0.700477
50.4014	15.0762	15.2077	3.85996	2.46303	2.23657	1.01363	0.717667
50.6008	15.1952	15.3415	3.86190	2.49642	2.29370	1.02524	0.744819
50.7169	15.4176	15.5337	3.89544	2.50221	2.37636	1.06042	0.782415
50.7252	15.5430	15.5890	3.92078	2.54885	2.37945	1.07910	0.785707
51.0262	15.7586	15.7239	4.01502	2.60783	2.38010	1.08630	0.795624
51.0320	15.7781	15.9859	4.03300	2.62802	2.38797	1.10281	0.827319
51.1450	15.8431	16.0903	4.07711	2.65142	2.46571	1.12916	0.840245
51.3659	15.9052	16.2039	4.07902	2.74138	2.54157	1.13385	0.840925
51.4052	15.9682	16.2109	4.08277	2.75622	2.60809	1.15478	0.934348
51.4269	16.1702	16.3555	4.09998	2.91932	2.69475	1.22065	0.960147
51.5428	16.1917	16.4832	4.22284	2.93856	2.77942	1.24519	0.975193
51.5956	16.3213	16.5410	4.24794	2.99895	2.83087	1.27757	1.05960
52.2596	16.3617	16.5823	4.38200	3.02685	2.96737	1.27823	1.06068
52.5924	16.4039	16.7497	4.44299	3.05247	3.00151	1.28976	1.08523
52.6608	16.4110	16.8407	4.46407	3.09840	3.04176	1.30046	1.10403
52.9824	16.8926	16.8961	4.48758	3.14470	3.05865	1.31220	1.12790
53.1298	17.0262	17.0560	4.60215	3.16543	3.11250	1.32460	1.13977
53.1325	17.0384	17.2581	4.64384	3.19272	3.18816	1.33310	1.23799
53.6187	17.0827	17.2885	4.70186	3.23432	3.28980	1.33356	1.35474
53.6239	17.1052	17.4130	4.71013	3.25650	3.29559	1.38284	1.36659
53.7249	17.5079	17.4471	4.74070	3.27081	3.36538	1.38790	1.41703
53.9606	17.5211	17.8841	4.74108	3.41478	3.38633	1.44969	1.41875
53.9746	17.6549	18.3035	4.81770	3.44942	3.46476	1.45014	1.43003
54.1109	18.2946	18.4772	4.81943	3.48108	3.46803	1.53039	1.43594
54.1235	18.4774	18.6002	4.83491	3.51267	3.49184	1.55451	1.49319
54.1372	18.8610	18.6787	4.89337	3.52330	3.57229	1.57256	1.53223
54.3043	19.4100	18.9194	4.91140	3.57235	3.59880	1.58309	1.57838

Table B.14 (cont.)

54.3590	19.5025	18.9655	5.01807	3.60641	3.71634	1.64416	1.58718
54.8197	19.5423	19.0575	5.02706	3.61602	3.73623	1.67799	1.72619
55.1536	19.8043	19.1285	5.18731	3.73331	3.75179	1.68987	1.74636
55.1553	19.8936	19.4147	5.28229	3.74266	3.78725	1.69017	1.75802
55.1781	19.9240	19.5523	5.28599	3.81424	3.80212	1.76291	1.78413
55.2630	19.9306	19.7325	5.36777	3.83136	3.80240	1.80139	1.79515
55.5420	19.9738	19.7485	5.39884	3.83732	3.83080	1.86413	1.79886
55.6402	20.0939	20.1799	5.40556	3.90106	3.85003	1.89363	1.81544
56.4648	20.1365	20.1842	5.49056	4.01456	3.88652	1.91689	1.86764
56.4962	20.4845	20.3765	5.51865	4.02154	4.05542	1.92316	2.04947
56.5838	20.6291	20.4696	5.54687	4.08672	4.09622	1.96570	2.06235
56.5903	20.9973	20.6158	5.64407	4.08958	4.25932	1.99711	2.09162
56.6531	20.9992	20.6818	5.69060	4.12954	4.26176	2.01679	2.15673
56.7654	21.0105	20.7166	5.78475	4.20695	4.29356	2.12370	2.16525
56.9525	21.1319	20.7841	5.80710	4.28010	4.34106	2.20957	2.16674
57.0809	21.1383	20.8770	5.88444	4.28751	4.34179	2.21467	2.17664
57.1097	21.7616	20.8830	5.93088	4.29430	4.34463	2.24930	2.19028
58.1522	21.8117	20.9173	6.03465	4.31760	4.44870	2.26393	2.20865
58.3717	21.8213	21.0549	6.08772	4.45039	4.49124	2.36151	2.37237
58.7271	21.8958	21.1565	6.12196	4.45313	4.49205	2.39122	2.39228
58.9779	21.9849	21.2609	6.44773	4.51952	4.51476	2.42250	2.48595
59.0946	22.0914	21.4574	6.48389	4.62613	4.59492	2.45751	2.49660
59.3230	22.8218	21.4622	6.73735	4.66059	4.68637	2.47231	2.61549
59.7647	22.9762	21.8628	6.86310	4.68014	4.72956	2.48123	2.67725
59.8422	23.6545	21.8797	6.98705	4.72333	4.74709	2.50547	2.67766
60.0636	23.8556	22.0684	7.00984	4.73107	4.78215	2.50858	2.69789
60.1404	23.9241	22.0727	7.05402	4.81930	4.90254	2.52523	2.72220
60.3473	23.9816	22.2688	7.07428	4.83965	4.93818	2.56456	2.79806
60.3524	24.0247	22.2703	7.09076	4.96176	4.95909	2.58247	3.10362
60.5108	24.0330	22.3059	7.13179	5.02684	5.03119	2.58339	3.17355
60.8491	24.2922	22.4143	7.21596	5.02769	5.07444	2.60171	3.20835
60.9570	24.3330	22.4328	7.32649	5.06297	5.08772	2.80751	3.28768
61.0446	24.3368	22.5103	7.42932	5.11229	5.16016	2.88341	3.34569
61.0987	24.4535	22.7083	7.48107	5.11566	5.16358	2.88352	3.41475
61.1518	24.7137	22.9362	7.56488	5.19778	5.16520	2.92147	3.46535
61.1624	24.7233	23.7492	7.57889	5.26304	5.17785	2.92510	3.47654
61.2334	24.7673	23.7715	7.60629	5.28497	5.23392	2.93113	3.50564
61.2364	25.0187	23.8016	7.62511	5.30045	5.24921	3.14519	3.54740
61.3304	25.0819	23.8374	7.63956	5.37046	5.28707	3.17324	3.61012
61.4250	25.2271	23.8618	7.74530	5.42394	5.30960	3.17733	3.63957
61.5671	25.2581	23.8644	7.84243	5.44543	5.40345	3.18149	3.65022
61.8929	25.2916	23.9604	7.99939	5.46319	5.41220	3.22393	3.65172
61.9457	25.5853	23.9911	8.16270	5.54513	5.43825	3.26402	3.69805
62.0357	25.6678	24.1884	8.18270	5.61751	5.46009	3.29117	3.78777
62.0451	25.7023	24.2240	8.24322	5.68926	5.55288	3.30780	3.78932
62.2899	25.7928	24.4154	8.27304	5.69669	5.62510	3.32335	3.87944
62.3904	25.9417	24.4654	8.33644	5.78950	5.69170	3.37584	3.89937
62.7748	25.9590	24.5540	8.45260	5.82620	5.70151	3.40875	3.92249

Table B.14 (cont.)

62.9061	26.1460	24.5745	8.55804	5.86780	5.74531	3.43322	3.96336
63.5176	26.2002	24.5900	8.57571	5.89036	5.75136	3.47286	3.96499
63.6713	26.2389	24.8056	8.58238	5.91766	5.75421	3.54930	4.01509
63.7634	26.4066	25.3010	8.72284	5.93131	5.90298	3.56185	4.02906
63.9088	26.4226	25.4662	8.97347	5.99621	6.01949	3.59578	4.04409
63.9687	26.9882	25.7726	9.00138	5.99969	6.10843	3.63531	4.04571
64.0533	27.1938	25.8300	9.12815	6.08243	6.18257	3.78595	4.08213
64.0839	27.4637	26.1992	9.30760	6.09313	6.21129	3.82909	4.34226
64.3131	27.6148	26.2277	9.42108	6.13688	6.27128	3.89033	4.37874
64.4313	27.8873	26.3233	9.46860	6.14093	6.37910	3.90836	4.40776
64.5492	28.0357	26.3427	9.56321	6.16998	6.43638	3.96012	4.41144
64.5672	28.2490	26.5435	9.59500	6.17270	6.44611	4.03329	4.42719
64.5882	28.4014	26.8631	9.67692	6.18862	6.57632	4.10379	4.43951
64.6702	28.5618	27.0551	9.72410	6.31455	6.61665	4.15990	4.46711
65.0434	28.7702	27.3486	9.84056	6.33785	6.62142	4.19128	4.51336
65.2278	28.9862	27.5012	9.85371	6.55064	6.67651	4.19726	4.52811
65.2535	29.1910	27.5198	9.94989	6.59949	6.85310	4.22359	4.56904
65.5508	29.2810	27.7118	9.95702	6.64661	7.03541	4.26022	4.57353
65.9005	29.3038	27.8190	9.96867	6.71416	7.03767	4.29139	4.61893
66.2234	29.3618	28.0616	10.0130	6.91171	7.17757	4.31695	4.73196
66.4179	29.5228	28.2334	10.1026	6.92160	7.25980	4.32253	4.87282
66.4666	29.7671	28.4113	10.2930	6.95786	7.29121	4.32493	4.92240
66.5959	29.8299	28.5881	10.3062	7.08335	7.33361	4.35268	4.95881
66.6663	29.8323	28.9228	10.4121	7.08366	7.33788	4.39427	5.06003
66.8803	29.8526	29.0525	10.5687	7.09579	7.36198	4.45068	5.10042
67.0612	30.0010	29.0829	10.6226	7.12515	7.54224	4.48540	5.10465
67.3666	30.1914	29.6355	10.6525	7.16482	7.60837	4.50157	5.21731
67.6207	30.2144	30.0195	10.6758	7.21772	7.63352	4.53773	5.27312
67.8479	30.2527	30.1801	10.7309	7.25555	7.63413	4.59824	5.27487
67.8828	30.3387	30.2143	10.8902	7.25907	7.68952	4.72532	5.28922
68.0676	30.4245	30.2556	10.9156	7.26899	7.74578	4.74607	5.32804
68.2083	30.4350	30.2639	10.9180	7.41898	7.85846	4.80918	5.49011
68.8202	30.6242	30.2704	11.0077	7.42760	7.87096	4.82991	5.51491
68.9773	30.7957	30.4869	11.0397	7.46790	7.89066	4.86293	5.52165
69.2985	31.3881	30.7822	11.0747	7.68236	7.89731	4.86312	5.62515
69.2996	31.5648	30.8188	11.1086	7.73410	7.96784	4.93248	6.06697
69.3870	31.7425	30.8208	11.1486	7.83345	7.97452	4.99906	6.29711
69.4236	31.7697	31.0618	11.1506	7.88607	8.01157	5.13052	6.30289
69.4757	31.7834	31.3131	11.1883	7.90532	8.14157	5.18677	6.31448
69.4986	31.8449	31.5148	11.1985	7.92780	8.21015	5.23270	6.35135
69.5408	31.8862	31.5423	11.2570	7.95313	8.23766	5.27787	6.41214
69.8660	32.0745	31.5998	11.3061	7.97142	8.28438	5.30727	6.43984
69.9272	32.1114	31.8078	11.3267	7.98666	8.35110	5.33084	6.56601
69.9916	32.1502	31.9339	11.5418	8.01938	8.59964	5.40807	6.59091
70.2693	32.1698	32.4913	11.8321	8.03826	8.62822	5.56647	6.59560
70.3862	32.4633	32.5633	11.9126	8.09865	8.74652	5.65355	6.59669
70.5197	32.5265	33.4817	11.9969	8.14975	8.86659	5.66797	6.62055
70.9900	32.5849	33.5847	12.1602	8.33137	8.87512	5.73387	6.72794

Table B.14 (cont.)

71.1198	32.6404	33.6309	12.2729	8.37944	8.94072	5.73390	6.77373
71.1258	32.7971	33.7101	12.4339	8.60877	8.99680	5.75217	6.77477
71.2610	33.0230	33.8563	12.5121	8.62848	9.13331	5.75355	6.80580
71.3860	33.1048	34.1533	12.5451	8.63056	9.22907	5.80306	6.86319
71.5427	33.1647	34.1750	12.5851	8.70312	9.30881	5.81296	6.97718
71.6201	33.3736	34.4179	12.5940	8.70598	9.78514	5.82921	6.98188
71.7234	33.4905	34.4789	12.6210	8.71242	9.91736	5.85416	6.99426
72.3419	33.8299	34.5923	12.6613	8.76413	9.94562	5.87008	7.00772
72.3697	34.0261	34.6180	12.6797	8.79538	10.1205	5.90370	7.11352
72.4787	34.1588	34.8803	12.7071	8.84582	10.1505	5.91819	7.16781
72.5834	34.2604	34.9217	12.7191	8.86085	10.2437	5.92832	7.17264
72.5877	34.2853	34.9773	13.0370	8.88809	10.2756	5.93454	7.17625
72.6543	34.3407	35.1362	13.5989	9.05534	10.3197	6.04842	7.19867
72.7767	34.4892	35.2736	13.7880	9.07425	10.4492	6.08097	7.23729
72.8115	34.7576	35.2763	14.1013	9.13811	10.4492	6.13851	7.31269
73.0890	34.7886	35.3790	14.1030	9.39548	10.7430	6.18650	7.37181
73.2729	34.8619	35.4943	14.3501	9.44603	10.7476	6.18684	7.43962
73.3714	35.1614	35.5609	14.3763	9.50734	10.7531	6.19587	7.51820
73.4134	35.2165	35.6641	14.4516	9.53601	10.9177	6.30242	7.56790
73.5184	35.3729	35.6986	14.4567	9.72244	10.9763	6.32569	7.68646
73.7031	35.5040	35.8698	14.5354	9.72396	11.0303	6.35453	7.69216
73.7613	35.5101	35.9223	14.5899	9.81449	11.1662	6.51151	7.83256
73.8688	35.8076	36.0188	14.7637	9.97364	11.1796	6.51171	7.84969
73.8946	35.9808	36.1734	15.0645	10.2740	11.1799	6.55383	7.85800
73.9101	36.2454	36.1832	15.1546	10.3336	11.3105	6.55614	8.00381
73.9400	36.5253	36.2359	15.5988	10.4703	11.3513	6.63663	8.04327
74.0128	36.5948	36.2767	15.7519	10.5040	11.5081	6.65759	8.07061
74.1469	36.7098	36.4970	15.7612	10.7927	11.5878	6.67590	8.14179
74.3628	36.7300	36.5799	15.8874	10.9017	11.6111	6.70024	8.20861
74.4654	36.9427	36.6175	15.9009	10.9743	11.6730	6.74352	8.21063
74.4833	36.9474	37.0336	15.9420	10.9830	11.6752	6.75118	8.26237
74.4993	36.9650	37.1049	16.0649	11.1369	11.7397	6.77695	8.32469
74.5091	37.1427	37.1182	16.2840	11.1449	11.8507	6.77714	8.36218
74.5989	37.5098	37.2156	16.4123	11.1583	12.0359	6.81909	8.42908
74.7648	37.5308	37.3153	16.6507	11.2014	12.0682	6.85816	8.43773
75.1102	37.7276	38.0882	16.7949	11.2471	12.1019	7.00722	8.47196
75.1168	37.7797	38.1302	16.8850	11.2519	12.2194	7.08379	8.47356
75.1201	37.8813	38.3437	16.9507	11.4493	12.3384	7.10787	8.64600
75.2160	38.1559	38.3872	17.0954	11.5801	12.3702	7.19481	8.74386
75.4786	38.1798	38.5493	17.2364	11.8892	12.4816	7.30652	8.84828
75.4896	38.2955	38.6929	17.4570	12.0689	12.4996	7.40771	8.91337
75.4939	38.6771	38.8469	17.8467	12.1261	12.6191	7.53756	8.97739
75.5001	38.8985	39.3824	18.2905	12.3416	12.7065	7.55175	9.06565
75.6076	38.9264	39.4445	18.3890	12.3594	12.7533	7.59110	9.14508
75.7063	38.9596	39.4763	18.4360	12.4965	12.9596	7.63890	9.15697
75.8696	39.2170	39.9595	18.4823	12.5601	13.0338	7.65973	9.18132
76.0674	39.4631	40.1971	18.6906	12.5897	13.1469	7.94737	9.19345
76.0755	40.8518	40.6904	19.4229	12.8201	13.1959	8.00902	9.22116

Table B.14 (cont.)

76.0960	40.8698	40.7358	19.7461	12.8218	13.2820	8.04841	9.32283
76.1774	41.4358	41.1081	19.7934	12.8904	13.7207	8.27034	9.38582
76.3565	41.5280	41.6853	20.1127	13.3414	13.7354	8.32260	9.47141
76.4691	41.7379	41.8920	20.1859	13.3705	13.7551	8.40124	9.50325
76.6317	41.9760	42.0831	20.1937	13.4673	13.8569	8.42835	9.64012
76.8347	42.6222	42.1311	20.2431	13.8088	14.1772	8.44198	9.64794
76.9680	42.7167	42.1630	20.3834	13.8970	14.6675	8.44385	9.70797
77.2943	42.7337	42.2134	20.8703	14.0673	14.7395	8.53934	9.82596
77.3365	42.7396	42.2779	21.0948	14.0904	14.7533	8.54110	9.87421
77.8148	42.8423	42.4591	21.1650	14.1353	15.1913	8.58322	9.92800
77.8456	42.9158	42.4685	21.2222	14.2515	15.3369	8.68369	9.98872
78.1003	42.9631	42.5551	21.5505	14.2976	15.3999	8.68679	10.1511
78.1401	43.2738	42.9010	21.6497	14.3873	15.4076	8.81576	10.1930
78.2672	43.4872	43.1622	21.8086	14.6146	15.6149	8.82622	10.2320
78.5149	43.5624	43.2158	21.8878	14.6654	15.6576	8.85141	10.3319
78.5370	43.7875	43.3336	22.3228	14.6868	15.8846	8.87598	10.3395
78.5386	43.8607	43.8147	22.9038	14.8143	15.9002	8.87973	10.3490
78.7331	44.0623	44.0219	23.4980	15.3319	15.9277	8.90175	10.5263
78.7769	44.1468	44.4060	23.5082	15.3506	15.9277	8.94427	10.6170
78.9505	44.2336	45.2064	23.7575	15.4958	16.3475	9.01717	10.7476
79.0166	45.0459	45.2074	23.8885	15.9657	16.3765	9.06431	10.7477
79.1426	45.4711	45.2298	24.0449	16.1351	16.4609	9.11334	10.8357
79.5280	45.5244	45.3755	24.0625	16.2346	16.4964	9.17232	11.2500
79.6307	45.9789	45.4701	24.0775	16.2442	16.8001	9.18975	11.5844
79.6356	46.3732	45.5586	24.2393	16.3055	16.8122	9.24962	11.6706
79.6474	46.6941	45.6674	24.3728	16.3350	17.0279	9.28424	11.7989
79.9923	47.0234	45.7965	24.4602	16.4231	17.2135	9.29835	11.8298
80.0539	47.2665	46.2316	24.8339	16.7475	17.4367	9.43972	11.8354
80.1377	47.7693	46.3750	24.8703	16.9784	17.6703	9.55596	11.8748
80.5821	47.8946	46.5028	24.8885	17.3490	17.6703	9.61564	11.8909
80.6612	47.9738	47.2165	24.9760	17.4607	17.7321	9.68574	12.0326
80.7403	48.2532	47.2201	25.0272	17.6977	17.9409	9.81957	12.1578
80.7631	48.2582	47.3539	25.8573	17.7307	17.9998	9.95613	12.3275
80.7682	48.3120	47.4519	26.0751	17.8280	18.5872	10.0260	12.4463
80.9245	49.1104	47.4635	26.2156	18.2857	18.6176	10.1049	12.5118
81.2432	49.3907	47.6439	26.2664	18.6140	18.9277	10.1530	12.5564
81.2925	49.4101	47.6744	27.2697	18.7989	19.1418	10.2872	12.6655
81.3077	49.5536	47.9735	27.2746	18.8300	19.1494	10.3453	12.8293
81.5501	49.6326	48.0120	27.6061	19.0146	19.1531	10.4003	12.8630
81.6916	49.7279	48.0554	27.7965	19.2460	19.2517	10.6159	12.8754
81.7863	50.4200	48.0648	27.8873	19.3527	19.3049	10.6171	12.9135
81.8021	50.5335	48.1141	28.0360	19.3965	19.3322	10.6263	13.0104
81.8855	50.6857	48.3902	28.1128	19.6370	19.5106	10.6869	13.0216
81.9053	50.7480	48.6486	28.5846	20.0385	19.6304	10.7981	13.0264
82.3868	50.8006	48.7058	28.6410	20.1041	20.1755	10.8605	13.3668
82.4257	51.0390	48.8885	28.7741	20.4239	21.2583	11.2088	13.4139
82.4313	51.1256	49.2286	28.8423	20.6675	21.3655	11.3873	13.4301
82.6491	51.1541	49.3357	28.8830	21.0016	21.4553	11.4211	13.7299

Table B.14 (cont.)

82.6861	51.1788	49.5249	29.1502	21.1644	21.7856	11.5095	13.7688
82.9644	51.2216	49.6003	29.1696	21.3739	22.0897	11.7339	14.0333
83.0284	51.2279	49.9877	30.3829	21.4184	22.3107	11.8370	14.2588
83.3819	51.3091	50.5451	30.4065	21.7285	22.3384	11.8692	14.3478
83.7461	51.4021	50.7551	30.7679	21.7652	22.6572	11.9189	14.4261
83.8977	51.4658	50.7901	31.2173	22.0662	22.7728	11.9575	14.4716
84.0577	51.5146	50.9975	31.4700	22.3924	23.1231	12.0156	14.6630
84.2383	51.5749	51.1708	32.0934	22.4317	23.4545	12.0305	14.8923
84.2593	52.1329	51.7338	32.3352	22.7088	23.4974	12.1258	14.8943
84.2968	52.5377	52.4495	32.8552	22.9727	23.5192	12.1628	14.9095
84.5187	52.8198	52.7805	32.9224	23.1942	23.8179	12.2069	15.2101
84.5748	52.8940	52.8874	33.3022	23.3739	24.2039	12.2259	15.3871
84.6998	53.3081	53.0561	33.9538	24.1292	24.2695	12.2741	15.4687
85.0152	53.4291	53.1297	34.4732	24.3390	24.4290	12.3330	15.6273
85.2772	53.4596	53.2892	34.4830	24.4139	24.5326	12.3636	15.6852
85.2790	53.8154	53.6652	34.8041	24.4916	25.0195	12.8614	15.9619
85.3014	53.8663	54.1254	34.8502	24.5314	25.1561	13.0262	16.2114
85.3826	53.9086	54.7096	37.6039	24.6676	25.6590	13.2575	16.2643
85.4621	53.9785	55.9163	37.6355	24.6693	25.7659	13.2945	16.2785
85.4973	54.0269	56.7739	37.7317	24.8161	27.2293	13.4195	16.5508
85.6714	54.6545	56.8069	37.7900	25.0302	27.4984	13.5764	16.7799
85.7645	54.6545	57.5181	38.6250	25.1756	28.1650	13.8678	16.7894
85.7779	55.1095	57.5828	39.0040	25.3396	28.5052	13.9461	17.5633
85.7861	55.1181	57.6303	39.4283	25.8923	30.8813	14.1032	17.8520
86.0229	55.2614	57.7910	39.8998	26.5006	31.0909	14.2395	18.0201
86.1336	55.4554	57.9313	39.9229	26.8622	31.8418	14.2860	18.0480
86.3856	55.9181	57.9368	40.6398	27.3657	32.0562	14.3183	18.1274
86.6223	56.0549	58.0047	41.2090	27.4263	32.1694	14.3733	18.4236
86.7964	56.9998	58.7515	41.4626	27.9606	32.5586	14.9235	18.5262
86.9171	57.2874	59.4611	41.9030	28.2903	32.9864	15.0725	18.6117
86.9934	57.3448	59.4872	41.9948	28.5670	33.9920	15.1610	19.0662
87.0336	57.4550	59.7420	43.0300	28.7298	34.1201	15.8257	19.1006
87.3286	57.9311	59.7687	43.7025	29.5035	34.4033	16.0238	19.3087
87.4075	58.3984	59.9023	44.0204	29.7030	34.6268	16.0524	19.3889
87.5169	58.5841	60.5300	46.1410	31.0944	34.6576	16.1484	19.4130
87.8525	58.8859	61.4191	46.7518	31.2315	34.7971	16.3958	19.4383
87.9743	59.4888	61.9379	47.3272	31.3610	35.0409	17.5379	19.6116
88.0274	59.7861	62.4801	47.5058	31.5578	35.1885	17.6417	19.7822
88.1166	60.0346	63.3716	47.7441	31.8001	36.1873	18.1146	19.9878
88.1930	60.2519	63.7093	48.3239	31.8693	36.2421	19.4795	20.1649
88.4137	60.6814	63.8084	48.4381	33.7332	36.3279	19.7974	20.2970
88.8369	60.7067	64.5337	48.7840	34.3236	37.2760	20.7849	20.3565
89.2502	61.1545	64.5995	49.1079	35.0648	37.7194	20.9243	20.7569
89.3748	61.6491	64.8158	50.5303	36.0830	38.1103	21.0068	20.8353
89.5386	61.8247	65.1337	51.6219	36.2119	40.4038	21.0366	20.9216
90.2771	62.2816	66.3508	51.8270	36.2977	40.4863	21.3544	21.2817
91.2654	62.7242	67.2904	52.1199	36.3316	41.5565	21.7802	21.3385
91.2735	63.1657	68.5835	53.0245	37.2075	42.5236	23.4827	21.4679

Table B.14 (cont.)

91.8150	65.0953	69.2138	53.6066	37.9817	45.1214	23.8211	22.2276
92.3452	66.3782	69.2743	54.7120	38.2547	45.1449	24.8613	22.2341
93.2548	67.1248	71.0903	54.7354	38.4111	45.7065	25.2382	22.2704
93.3121	67.9187	71.3343	55.0006	39.0135	47.3477	25.3660	22.6007
93.5742	69.2978	73.4163	55.5474	39.1539	48.2875	25.9992	22.6368
93.6727	70.1076	74.6023	56.3704	39.3387	49.4727	26.0892	22.7475
94.0990	72.7325	75.8095	57.8244	40.0561	49.4828	26.4776	23.2817
94.6344	72.7995	76.0920	57.8729	40.5387	54.5723	26.9839	23.4619
94.7754	73.4005	79.6000	59.3213	41.0256	56.8091	27.9057	24.0414
94.8403	73.4096	80.9124	60.9561	41.7767	60.0593	31.1907	25.5114
94.8473	73.8988	82.4866	63.8284	42.5115	61.4675	34.4807	25.5365
95.9588	74.1758	82.8571	65.1560	46.5513	63.1287	34.5648	26.4404
96.1140	74.4704	84.7380	65.4198	53.2279	65.8872	35.3630	30.3174
96.3460	75.6036	84.7575	70.7919	59.5764	66.8924	44.3234	31.1362
96.3463	75.9105	85.8855	72.0787	59.7986	68.8586	44.5799	31.9113
96.8704	80.5891	92.2627	78.7329	64.9234	71.9763	51.7569	33.1842
97.0917	80.6800	93.4703	83.1832	72.9655	80.4752	55.7215	37.3812
97.2467	82.1659	94.6602	87.5267	77.1126	80.6623	56.7375	73.3154
97.4269	86.7862	94.6993	90.4428	86.3259	84.2255	74.0059	96.3658

Table B.15 Raw data of the histograms illustrating the distributions of SCGE % tail DNA values from the CHO cell negative controls at 0 and 24 hour liquid-holding recovery.

LH_0h	LH_24h
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
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Table B.15 (cont.)

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Table B.15 (cont.)

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0	0
0.000219	0
0.000243	0
0.000298	0
0.000512	0
0.000597	0
0.000614	0
0.000638	0
0.000679	0
0.000710	0
0.000778	0
0.000821	0
0.000894	0
0.001035	0
0.001178	0

Table B.15 (cont.)

0.001187	0
0.001314	0
0.001357	0
0.001369	0
0.001386	0
0.001395	0
0.001413	0
0.001424	0
0.001480	0
0.001481	0
0.001545	0
0.001566	0
0.001599	0
0.001624	0
0.001654	0
0.001664	0
0.001666	0
0.001673	0
0.001709	0
0.001744	0
0.001792	0
0.001865	0
0.001885	0
0.001908	0
0.002191	0
0.002254	0
0.002277	0
0.002367	0
0.002413	0
0.002470	0
0.002544	0
0.002686	0
0.002715	0
0.002879	0
0.002915	0
0.002955	0
0.002974	0
0.002977	0
0.003121	0
0.003141	0
0.003209	0
0.003291	0
0.003326	0
0.003461	0
0.003468	0
0.003606	0
0.003870	0
0.004097	0

Table B.15 (cont.)

0.004142	0
0.004165	0
0.004238	0
0.004242	0
0.004264	0
0.004296	0
0.004401	0
0.004411	0
0.004420	0
0.004495	0
0.004505	0
0.004578	0
0.004612	0
0.004678	0
0.004717	0
0.004892	0
0.005188	0
0.005520	0
0.005679	0
0.005680	0
0.005886	0
0.005889	0
0.005908	0
0.005971	0
0.006099	0
0.006349	0
0.006597	0
0.006654	0
0.006660	0
0.006697	0
0.006719	0
0.006745	0
0.006881	0
0.006901	0
0.006983	0
0.006995	0
0.007033	0
0.007107	0
0.007354	0
0.007504	0
0.007555	0
0.007678	0
0.007866	0
0.008012	0
0.008018	0.000543
0.008121	0.000594
0.008124	0.000729
0.008505	0.000731

Table B.15 (cont.)

0.008573	0.000783
0.008574	0.000797
0.008663	0.000903
0.008755	0.001061
0.008956	0.001190
0.009219	0.001246
0.009399	0.001262
0.009427	0.001308
0.009443	0.001424
0.009459	0.001424
0.009543	0.001463
0.009556	0.001466
0.009581	0.001482
0.009724	0.001494
0.009729	0.001519
0.009845	0.001523
0.010224	0.001536
0.010503	0.001570
0.010673	0.001609
0.010679	0.001612
0.010873	0.001683
0.010906	0.001733
0.011075	0.001762
0.011105	0.001763
0.011291	0.001771
0.011716	0.001779
0.011797	0.001839
0.011805	0.001928
0.011889	0.001957
0.012331	0.001973
0.013132	0.002004
0.013589	0.002020
0.013917	0.002046
0.014531	0.002047
0.014584	0.002096
0.014625	0.002119
0.015036	0.002261
0.015183	0.002328
0.015967	0.002437
0.016105	0.002477
0.016129	0.002510
0.016411	0.002542
0.017003	0.002631
0.017047	0.002779
0.017078	0.002998
0.017256	0.003004
0.017624	0.003006
0.018611	0.003008

Table B.15 (cont.)

0.018783	0.003161
0.018928	0.003259
0.019323	0.003283
0.019485	0.003290
0.020311	0.003347
0.020604	0.003353
0.021064	0.003472
0.021280	0.003497
0.021548	0.003654
0.022214	0.003751
0.022386	0.003783
0.022429	0.003974
0.022954	0.004222
0.023032	0.004228
0.023053	0.004354
0.023506	0.004364
0.023614	0.004375
0.023897	0.004412
0.025117	0.004487
0.025138	0.004505
0.025231	0.004542
0.025553	0.004556
0.025574	0.004574
0.025771	0.004924
0.025811	0.004938
0.025871	0.005168
0.026079	0.005178
0.026330	0.005305
0.026430	0.005497
0.026446	0.005622
0.026932	0.005649
0.026964	0.005680
0.027211	0.005923
0.028106	0.005926
0.028204	0.006015
0.028300	0.006054
0.028966	0.006091
0.029439	0.006316
0.029553	0.006337
0.029892	0.006527
0.029986	0.006787
0.030641	0.007134
0.031404	0.007399
0.031893	0.007439
0.032785	0.007701
0.033155	0.007786
0.033276	0.007793
0.034051	0.007822

Table B.15 (cont.)

0.035824	0.007880
0.036195	0.008273
0.036222	0.008289
0.036366	0.008550
0.036845	0.009227
0.036932	0.009300
0.037052	0.009451
0.037071	0.009468
0.037239	0.009504
0.037508	0.009573
0.037718	0.009674
0.039359	0.009704
0.039465	0.009795
0.039923	0.010218
0.040749	0.010238
0.041341	0.010570
0.041878	0.010762
0.042031	0.010825
0.042568	0.010890
0.042767	0.010896
0.043718	0.011134
0.043908	0.011215
0.044854	0.011334
0.044898	0.011420
0.045145	0.011446
0.045844	0.011491
0.045884	0.011580
0.046123	0.011739
0.046144	0.011795
0.046165	0.012459
0.047329	0.012489
0.047758	0.012868
0.047819	0.012876
0.047825	0.012916
0.048010	0.013084
0.048648	0.013200
0.049078	0.013204
0.049355	0.013257
0.049402	0.013502
0.051138	0.013762
0.051595	0.013815
0.052225	0.013860
0.052247	0.014109
0.054464	0.014111
0.054914	0.014153
0.054925	0.014211
0.055465	0.014312
0.055491	0.014434

Table B.15 (cont.)

0.056141	0.014561
0.057336	0.015022
0.057623	0.015096
0.057874	0.015201
0.057918	0.015668
0.058688	0.015883
0.059431	0.016124
0.059447	0.017107
0.059558	0.017252
0.060042	0.017378
0.060117	0.017461
0.060146	0.017710
0.060850	0.017833
0.060865	0.018466
0.061128	0.018936
0.061698	0.018981
0.061801	0.019098
0.062198	0.019166
0.062803	0.019376
0.063381	0.019438
0.063777	0.019966
0.064171	0.020560
0.064306	0.020595
0.066729	0.020685
0.067414	0.021031
0.068357	0.021167
0.068597	0.021286
0.068844	0.021461
0.070515	0.021699
0.070640	0.021726
0.071449	0.021804
0.072451	0.022314
0.072851	0.023094
0.072896	0.023106
0.073140	0.023133
0.073201	0.023332
0.073649	0.023505
0.073783	0.023585
0.074381	0.024197
0.075523	0.025052
0.075567	0.025531
0.075806	0.025559
0.076199	0.025639
0.077550	0.025771
0.077784	0.025908
0.078338	0.026455
0.078769	0.026777
0.078887	0.027559

Table B.15 (cont.)

0.079060	0.027578
0.079818	0.028161
0.080851	0.028741
0.081027	0.028945
0.081218	0.029008
0.081286	0.029113
0.081650	0.029609
0.082448	0.029816
0.082953	0.030475
0.082966	0.030679
0.083445	0.031131
0.084214	0.031421
0.084319	0.031707
0.084375	0.031707
0.085355	0.031828
0.085721	0.032208
0.086807	0.032453
0.087556	0.033164
0.088708	0.033295
0.089544	0.033702
0.091574	0.033795
0.091663	0.034284
0.091864	0.034293
0.094076	0.034694
0.094857	0.035087
0.095503	0.035213
0.096780	0.035731
0.097121	0.036529
0.099159	0.036682
0.099721	0.037298
0.100905	0.037478
0.100920	0.038118
0.101666	0.038173
0.102942	0.038406
0.103602	0.038430
0.103953	0.038476
0.104083	0.038620
0.104830	0.038861
0.105604	0.038898
0.105626	0.039057
0.106803	0.039308
0.109044	0.039524
0.109515	0.039716
0.110946	0.039982
0.111857	0.040000
0.112043	0.040010
0.112286	0.040399
0.112596	0.040751

Table B.15 (cont.)

0.113124	0.040803
0.113261	0.041559
0.113565	0.044493
0.114127	0.044619
0.115066	0.045456
0.115602	0.046306
0.117960	0.046518
0.118229	0.047132
0.118337	0.047140
0.121196	0.048112
0.121473	0.048503
0.122046	0.048705
0.122454	0.051301
0.122783	0.051822
0.123193	0.051889
0.127110	0.052199
0.127207	0.052292
0.127697	0.052322
0.128534	0.052676
0.128790	0.052713
0.128963	0.052834
0.131670	0.052896
0.132621	0.053234
0.135243	0.054582
0.135509	0.054583
0.137728	0.054586
0.138616	0.054662
0.139851	0.055538
0.140569	0.056152
0.141670	0.056184
0.143732	0.056345
0.143963	0.056458
0.144043	0.056651
0.144110	0.057429
0.144239	0.057614
0.146796	0.057872
0.148093	0.058114
0.148381	0.058916
0.149862	0.059005
0.150008	0.059217
0.156791	0.059842
0.157032	0.060034
0.158750	0.060189
0.160278	0.060202
0.160592	0.060662
0.161093	0.062376
0.162558	0.062682
0.163115	0.064077

Table B.15 (cont.)

0.164129	0.064241
0.164497	0.064367
0.165151	0.064717
0.165186	0.064776
0.166116	0.065609
0.166973	0.065613
0.167546	0.065675
0.168339	0.065822
0.171435	0.065987
0.172121	0.066061
0.175949	0.066662
0.177050	0.066800
0.180027	0.067024
0.182865	0.067704
0.185458	0.067727
0.186162	0.067878
0.189565	0.068267
0.191164	0.069050
0.191853	0.069139
0.198354	0.069658
0.199134	0.070496
0.199237	0.071835
0.200769	0.071877
0.203195	0.071986
0.203242	0.072059
0.206374	0.073001
0.207773	0.073160
0.208078	0.073205
0.209531	0.074154
0.210652	0.074265
0.215861	0.074309
0.219277	0.074738
0.223083	0.075928
0.223157	0.076888
0.223628	0.077170
0.225323	0.077193
0.225575	0.077888
0.226958	0.078352
0.227953	0.079307
0.228897	0.079877
0.229189	0.080053
0.229321	0.080344
0.230028	0.081310
0.230511	0.081438
0.239532	0.081979
0.240046	0.082389
0.246735	0.084498
0.248102	0.085430

Table B.15 (cont.)

0.248938	0.086139
0.250098	0.086303
0.252298	0.087356
0.252841	0.087516
0.256148	0.089627
0.268011	0.091115
0.268565	0.091348
0.268921	0.091572
0.269455	0.092177
0.271275	0.093418
0.273467	0.093736
0.274030	0.094655
0.277540	0.096108
0.278202	0.096195
0.281207	0.096450
0.283229	0.097656
0.285170	0.097867
0.287215	0.098197
0.289839	0.098247
0.290728	0.098472
0.293510	0.099115
0.293912	0.099286
0.294073	0.099352
0.294771	0.099508
0.295303	0.101262
0.295546	0.102289
0.296824	0.104347
0.297113	0.104441
0.301883	0.104504
0.302515	0.104866
0.303890	0.105014
0.316595	0.105695
0.319923	0.105891
0.320681	0.107221
0.322410	0.107554
0.327815	0.109358
0.328851	0.109765
0.329095	0.109963
0.330321	0.110516
0.332088	0.111779
0.333606	0.112654
0.334463	0.113270
0.338402	0.113749
0.339170	0.114843
0.339624	0.115239
0.341468	0.115770
0.342313	0.116291
0.342362	0.116549

Table B.15 (cont.)

0.347924	0.117754
0.349969	0.119218
0.353864	0.119528
0.355399	0.119576
0.356132	0.120090
0.358084	0.120829
0.360443	0.120924
0.360761	0.122718
0.361423	0.123321
0.363777	0.123986
0.363888	0.124124
0.364371	0.129080
0.364759	0.129255
0.365253	0.129276
0.375427	0.130176
0.378928	0.130582
0.380042	0.130666
0.381138	0.130679
0.381718	0.131649
0.386081	0.132770
0.389160	0.133153
0.394341	0.133288
0.395630	0.134345
0.397009	0.134763
0.397452	0.134818
0.399273	0.136460
0.399566	0.137232
0.400580	0.138714
0.411755	0.138969
0.412762	0.139305
0.414645	0.140469
0.414778	0.141752
0.415347	0.143314
0.415995	0.146110
0.417922	0.147002
0.425037	0.147796
0.425758	0.149197
0.429769	0.151252
0.432113	0.151660
0.436232	0.152464
0.437453	0.153121
0.439028	0.154442
0.444178	0.155214
0.447467	0.155916
0.449036	0.156024
0.449545	0.156419
0.451474	0.157376
0.451960	0.157668

Table B.15 (cont.)

0.454593	0.161599
0.456999	0.162455
0.460157	0.163972
0.470385	0.164484
0.472249	0.164499
0.472342	0.165391
0.474649	0.165846
0.476899	0.166001
0.477595	0.166981
0.484948	0.168244
0.488161	0.168722
0.489263	0.169063
0.496753	0.169349
0.507108	0.169519
0.510647	0.170110
0.516517	0.170656
0.517311	0.170672
0.517876	0.171080
0.520420	0.172606
0.523700	0.172608
0.524206	0.174419
0.525618	0.175536
0.525675	0.175579
0.540651	0.176207
0.542797	0.176610
0.544720	0.176969
0.545270	0.179353
0.545885	0.181064
0.550330	0.181174
0.550576	0.182072
0.553503	0.183778
0.565571	0.184131
0.570753	0.187012
0.571356	0.189961
0.573682	0.191157
0.588379	0.191187
0.593627	0.191653
0.594499	0.191917
0.600898	0.192307
0.609139	0.193253
0.611696	0.193529
0.620858	0.194919
0.634120	0.196364
0.647373	0.196879
0.648148	0.198056
0.668980	0.202915
0.671373	0.204190
0.675018	0.204380

Table B.15 (cont.)

0.684049	0.206788
0.686188	0.207829
0.692956	0.209995
0.699340	0.210506
0.705882	0.217403
0.708405	0.218741
0.708496	0.219594
0.708546	0.221888
0.710063	0.227846
0.716209	0.233055
0.720531	0.233670
0.725149	0.234726
0.725822	0.235567
0.729860	0.238264
0.732610	0.239010
0.741308	0.242047
0.746800	0.242579
0.747586	0.245449
0.752259	0.253112
0.759451	0.253675
0.764492	0.254848
0.765183	0.257830
0.773638	0.258030
0.774047	0.260788
0.776756	0.264092
0.777310	0.264634
0.778384	0.272494
0.779620	0.275182
0.785044	0.276425
0.788328	0.277644
0.793842	0.280455
0.794467	0.281064
0.794910	0.281223
0.801136	0.283567
0.806699	0.284303
0.808106	0.284547
0.808973	0.291746
0.818535	0.293539
0.822594	0.295177
0.828298	0.296713
0.828316	0.303673
0.842360	0.303866
0.854767	0.306338
0.854819	0.308884
0.854890	0.311945
0.863092	0.312719
0.864893	0.316567
0.869624	0.319352

Table B.15 (cont.)

0.874905	0.319764
0.877005	0.319891
0.877582	0.321952
0.877898	0.325778
0.880216	0.325789
0.882027	0.326697
0.894425	0.329032
0.903276	0.332226
0.917706	0.334668
0.920856	0.336929
0.921196	0.338247
0.929533	0.339381
0.932866	0.340232
0.936713	0.341305
0.936915	0.342920
0.937415	0.345168
0.938070	0.351422
0.938326	0.359857
0.951127	0.360388
0.954578	0.363661
0.958669	0.365250
0.961937	0.367123
0.976283	0.368731
0.986106	0.371292
0.988505	0.372542
0.997753	0.380071
0.999381	0.383258
1.03666	0.386828
1.04170	0.387710
1.04178	0.391329
1.04247	0.395207
1.05475	0.398099
1.05877	0.400199
1.07574	0.400887
1.07813	0.401595
1.08229	0.402977
1.08910	0.406555
1.09914	0.407001
1.10240	0.415564
1.11453	0.426807
1.11788	0.427780
1.12605	0.431482
1.13291	0.438656
1.14397	0.440614
1.14609	0.442194
1.14707	0.445457
1.14793	0.447229
1.15010	0.449366

Table B.15 (cont.)

1.15153	0.455374
1.15998	0.475612
1.16204	0.485623
1.16402	0.487054
1.17777	0.488194
1.17840	0.489341
1.18232	0.498104
1.18245	0.505100
1.18269	0.508988
1.20072	0.516557
1.20341	0.521779
1.20952	0.522365
1.21710	0.524113
1.21932	0.525056
1.22743	0.530885
1.22971	0.536227
1.23531	0.538343
1.23882	0.540455
1.23978	0.544606
1.24291	0.550150
1.24765	0.554091
1.25434	0.558636
1.26162	0.562157
1.26745	0.566738
1.26917	0.570302
1.27482	0.572941
1.28943	0.580395
1.29790	0.582243
1.29920	0.590733
1.31132	0.597408
1.31579	0.605225
1.32879	0.605271
1.33233	0.606587
1.36192	0.620076
1.36281	0.629947
1.36565	0.636261
1.37147	0.644558
1.37517	0.644673
1.38955	0.651159
1.40247	0.651393
1.42950	0.653297
1.43226	0.653462
1.43938	0.660361
1.44309	0.666558
1.44480	0.667992
1.46188	0.670752
1.46827	0.685026
1.47074	0.687300

Table B.15 (cont.)

1.48730	0.691901
1.50565	0.701023
1.50587	0.702203
1.53674	0.706803
1.53687	0.708543
1.53901	0.714955
1.54514	0.716459
1.55493	0.723669
1.56256	0.724317
1.56691	0.726023
1.58818	0.728696
1.59470	0.729498
1.60436	0.735080
1.60701	0.736733
1.61153	0.739252
1.62091	0.745305
1.62827	0.757901
1.63232	0.762532
1.63719	0.773159
1.64534	0.782431
1.64686	0.783961
1.65354	0.788072
1.65838	0.791514
1.67222	0.802201
1.67321	0.803431
1.69158	0.816017
1.70238	0.820973
1.71446	0.828808
1.71750	0.837489
1.72305	0.839205
1.72460	0.840366
1.72947	0.846207
1.74651	0.850257
1.75447	0.853375
1.77455	0.866694
1.77631	0.879364
1.77633	0.882163
1.77875	0.883452
1.79720	0.898202
1.79810	0.900036
1.80052	0.902486
1.82315	0.942991
1.82639	0.949425
1.83038	0.961081
1.83944	0.965370
1.84546	0.968838
1.85266	0.973111
1.88540	0.977345

Table B.15 (cont.)

1.88585	0.981165
1.89713	0.982471
1.90461	0.984658
1.92809	0.987235
1.92852	0.991798
1.92865	0.992612
1.92877	0.993274
1.93821	1.00276
1.94462	1.00741
1.94489	1.01211
1.94749	1.02223
1.95240	1.02279
1.96384	1.03760
1.97107	1.04725
1.97396	1.05638
1.98050	1.06562
1.98063	1.07093
1.98459	1.07668
1.98946	1.07746
1.99374	1.09710
2.00859	1.09778
2.01339	1.10071
2.02735	1.10790
2.04422	1.13423
2.08072	1.14136
2.09017	1.14553
2.10017	1.14882
2.10558	1.15411
2.12020	1.17881
2.12660	1.18120
2.13318	1.18268
2.14144	1.19090
2.14466	1.19427
2.14840	1.20059
2.15566	1.20134
2.15805	1.20896
2.15959	1.21861
2.16196	1.22700
2.16376	1.23134
2.16425	1.23726
2.16729	1.23727
2.16738	1.27163
2.16937	1.27721
2.18072	1.27938
2.19684	1.30245
2.20508	1.30330
2.21022	1.31810
2.21024	1.33208

Table B.15 (cont.)

2.21790	1.34663
2.22143	1.34903
2.23100	1.35071
2.23692	1.36403
2.24125	1.36406
2.28895	1.37672
2.28905	1.38120
2.29979	1.39306
2.30240	1.39615
2.31114	1.39650
2.31717	1.41565
2.32557	1.41727
2.33950	1.43427
2.33969	1.43728
2.34138	1.44074
2.35844	1.44314
2.36707	1.45261
2.37200	1.46551
2.38586	1.47459
2.38643	1.47698
2.40708	1.50125
2.43993	1.50164
2.44455	1.51096
2.44716	1.52870
2.45027	1.53846
2.46117	1.55497
2.46314	1.55839
2.48634	1.57515
2.50669	1.57815
2.52727	1.62223
2.54687	1.64467
2.56493	1.64658
2.56879	1.65952
2.57179	1.66257
2.57353	1.67317
2.57628	1.68247
2.58301	1.68794
2.59071	1.69148
2.59168	1.71601
2.61189	1.71620
2.63027	1.72974
2.64082	1.74852
2.67382	1.74938
2.68115	1.74988
2.68565	1.78039
2.68879	1.79056
2.70635	1.79235
2.71098	1.79685

Table B.15 (cont.)

2.71170	1.80040
2.73125	1.81621
2.73792	1.82944
2.77246	1.83450
2.77757	1.83744
2.78148	1.85373
2.78747	1.87335
2.80451	1.87510
2.80566	1.87962
2.82129	1.88192
2.87661	1.89209
2.87974	1.89504
2.88026	1.89545
2.88213	1.90568
2.90056	1.91045
2.90098	1.92994
2.91543	1.96619
2.94160	1.96941
2.97965	1.97556
3.00018	1.97716
3.00688	1.98694
3.05919	1.99299
3.06816	1.99780
3.06964	1.99789
3.07794	2.02691
3.09134	2.03071
3.11700	2.06114
3.17133	2.08720
3.18857	2.11927
3.19834	2.14699
3.21351	2.17651
3.22294	2.18464
3.23019	2.19709
3.24696	2.20076
3.26274	2.22399
3.26597	2.23932
3.27139	2.24048
3.27197	2.26137
3.27784	2.26552
3.28087	2.28484
3.28504	2.28895
3.29057	2.29534
3.31515	2.30150
3.31906	2.30392
3.31982	2.32293
3.32448	2.33423
3.34244	2.34452
3.34831	2.35059

Table B.15 (cont.)

3.35467	2.35439
3.35570	2.36046
3.37918	2.38240
3.40160	2.38330
3.40930	2.39821
3.41688	2.40734
3.48596	2.44464
3.48895	2.45071
3.49095	2.47937
3.52633	2.48728
3.53197	2.48823
3.54063	2.57321
3.56525	2.57384
3.58170	2.58327
3.59035	2.58578
3.63342	2.59352
3.63590	2.63038
3.65281	2.63210
3.65601	2.65470
3.65957	2.66340
3.67007	2.69159
3.67274	2.70172
3.68123	2.71343
3.68996	2.71672
3.69506	2.71944
3.71035	2.72481
3.72390	2.76308
3.75557	2.76601
3.75678	2.77729
3.76343	2.78900
3.76774	2.80145
3.83821	2.81064
3.84555	2.84393
3.84642	2.86359
3.85501	2.88505
3.85895	2.91133
3.86675	2.93281
3.88687	2.94935
3.89528	2.95396
3.90666	2.98877
3.93014	2.99464
3.94091	3.03409
3.99476	3.05281
3.99864	3.06847
4.00121	3.08886
4.00455	3.09056
4.02318	3.10455
4.03774	3.12146

Table B.15 (cont.)

4.04187	3.14046
4.05310	3.15132
4.08937	3.17226
4.09053	3.17578
4.09635	3.17664
4.10275	3.18538
4.13238	3.26106
4.13322	3.26914
4.14389	3.27026
4.16315	3.28763
4.21243	3.28794
4.22156	3.29378
4.23825	3.30506
4.26454	3.30816
4.27656	3.30846
4.28646	3.33045
4.29695	3.34087
4.30487	3.35740
4.31020	3.35868
4.31462	3.41207
4.32388	3.43169
4.32764	3.44364
4.33313	3.48312
4.34646	3.53062
4.35374	3.53711
4.39220	3.54013
4.42893	3.61216
4.44518	3.62261
4.44804	3.62542
4.44819	3.63567
4.45465	3.63874
4.45872	3.64502
4.47841	3.67249
4.48058	3.68691
4.53582	3.71148
4.55263	3.71505
4.55742	3.76792
4.56925	3.77286
4.58061	3.79191
4.60136	3.80886
4.61539	3.81002
4.62939	3.85050
4.63878	3.86053
4.67955	3.86843
4.69141	3.89377
4.70198	3.89387
4.73681	3.90831
4.75144	3.92192

Table B.15 (cont.)

4.76158	3.93870
4.78555	3.94493
4.78682	3.95310
4.80287	4.01578
4.83302	4.04150
4.84666	4.15948
4.85970	4.17927
4.87686	4.19633
4.90724	4.22692
4.93440	4.23247
4.94856	4.30054
4.97676	4.31377
4.98396	4.31698
5.08309	4.36701
5.12025	4.40503
5.14921	4.41927
5.18262	4.42108
5.21786	4.43403
5.21855	4.45275
5.22094	4.47508
5.22410	4.48954
5.24001	4.49865
5.24157	4.50803
5.25823	4.53864
5.27664	4.58392
5.30151	4.62407
5.32217	4.62781
5.35411	4.63306
5.39721	4.63765
5.40866	4.64576
5.44693	4.66668
5.44768	4.66858
5.51883	4.68410
5.52811	4.71183
5.55903	4.72058
5.58371	4.75172
5.60619	4.75510
5.61166	4.76599
5.64390	4.78784
5.68989	4.85832
5.69451	4.91227
5.72520	4.94118
5.73588	4.94905
5.80174	4.96838
5.81327	4.97445
5.82748	4.97880
5.83349	4.98447
5.84091	5.03277

Table B.15 (cont.)

5.84237	5.05562
5.89789	5.07338
5.91893	5.07630
5.92660	5.14385
5.92944	5.17318
5.93564	5.19353
5.99437	5.20936
5.99531	5.25697
6.01211	5.28985
6.08121	5.32252
6.21076	5.36480
6.21172	5.36703
6.23183	5.41021
6.23694	5.47282
6.31809	5.47646
6.58728	5.49494
6.60186	5.50113
6.78246	5.57267
6.85500	5.61472
6.86952	5.64700
6.87510	5.66548
6.88985	5.72149
6.96518	5.77249
6.98097	5.87844
6.99158	5.88567
7.00222	5.89603
7.01249	5.95463
7.02585	5.95959
7.05483	5.98444
7.08101	6.00465
7.10049	6.05389
7.15952	6.05490
7.16022	6.17190
7.22283	6.18711
7.24554	6.20815
7.24896	6.28173
7.36540	6.42628
7.41849	6.44078
7.43803	6.47672
7.44807	6.50351
7.46510	6.70620
7.53938	6.71839
7.56626	6.85834
7.57361	6.95578
7.57913	7.08287
7.61601	7.08854
7.63247	7.13627
7.77363	7.21137

Table B.15 (cont.)

7.98490	7.28897
7.98591	7.32764
8.02483	7.33637
8.10732	7.38271
8.11081	7.44342
8.14372	7.44716
8.16358	7.49054
8.19726	7.51487
8.20815	7.65637
8.37421	7.67532
8.42923	7.68751
8.44353	7.83181
8.51534	7.95237
8.82465	8.01738
8.82779	8.26818
8.91523	8.41198
8.91754	8.81436
9.04253	9.15106
9.05932	9.25003
9.16251	9.33579
9.28685	9.39268
9.31819	9.43585
9.36587	9.66910
9.36732	9.69412
9.41786	9.74678
9.48831	9.75708
9.61363	10.1151
9.74043	10.1944
10.0519	10.2765
10.3461	10.3273
10.5828	10.3966
11.0333	10.5426
11.0680	10.9301
11.1549	11.0227
11.2485	11.0944
11.7927	11.2011
11.8414	11.3331
12.0497	11.6830
12.0969	11.7440
12.7283	11.8056
13.1326	11.8443
13.4714	11.8685
13.6698	11.8996
13.6819	11.9855
14.0386	12.2743
14.4649	12.6474
14.5604	12.9483
19.8591	13.0902

Table B.15 (cont.)

24.1797	13.2244
31.6988	17.5669
37.5840	18.5837
44.3790	21.0268
63.5732	83.3272
99.7088	86.2647

Table B.16 Raw data of the plating efficiency of HAA-treated CHO cells. The blank-corrected absorbency values were normalized to the concurrent negative control (%).

	Negative control	CAA			BAA			IAA		
		5 mM	6 mM	7 mM	30 µM	60 µM	90 µM	15 µM	25 µM	35 µM
	107.370	68.730	59.478	38.254	90.136	68.549	63.107	48.776	40.068	41.338
	95.034	56.395	60.930	42.426	89.955	69.093	68.186	43.515	41.156	32.812
	97.029	57.846	58.027	41.519	75.442	59.478	48.050	52.222	35.170	30.998
	100.295	58.571	54.580	46.961	84.694	64.376	39.161	37.166	31.905	27.551
	97.211	61.837	64.376	38.073	82.517	57.664	58.209	49.683	33.719	31.179
	96.848	57.483	51.134	36.077	64.014	51.678	27.370	32.086	36.440	31.723
	106.644	62.562	64.014	60.023	74.535	57.664	42.063	48.776	50.590	43.878
	99.569	61.111	50.952	46.236	50.952	37.710	37.166	45.692	60.023	67.823
	99.310	43.941	54.426	50.747	81.835	73.005	58.105	72.269	39.710	49.828
	102.621	48.172	47.252	48.724	72.453	70.798	65.095	86.618	59.945	46.700
	98.942	52.035	57.370	53.139	91.032	82.203	65.279	83.307	62.152	39.526
	98.942	52.955	52.771	56.634	93.976	77.604	61.784	81.283	47.804	40.814
	99.862	51.851	59.761	56.266	93.976	87.905	73.741	90.297	57.737	53.874
	99.126	50.195	55.714	47.252	79.076	73.189	63.072	87.905	68.039	53.507
	95.263	48.724	59.945	61.232	89.377	83.123	65.831	92.504	73.741	51.667
	105.932	45.413	53.691	55.162	85.146	95.999	75.765	86.802	49.460	43.389
	101.958	61.701	54.958	43.654	105.330	94.621	87.085	99.380	98.190	57.933
	88.275	51.587	51.587	49.603	82.920	78.161	79.747	88.076	88.870	64.279
	100.967	57.734	52.380	47.620	88.671	83.317	77.962	76.574	82.127	77.169
	93.827	50.793	50.397	49.207	94.422	79.549	86.093	77.566	72.608	57.933
	102.355	63.684	55.354	48.413	88.473	84.507	81.730	82.127	72.608	61.502
	101.363	61.502	52.181	42.861	89.663	84.705	75.186	100.768	97.595	50.397
	109.693	59.916	61.899	54.760	97.000	86.490	74.591	92.638	80.937	76.177
	101.562	60.511	60.114	47.025	97.199	83.515	75.979	74.988	69.633	60.511
number	24	24	24	24	24	24	24	24	24	24
Average	100.000	56.052	55.970	48.411	85.116	74.371	64.598	72.126	60.426	49.688
SE	0.954	1.300	0.937	1.382	2.379	2.883	3.268	4.332	4.141	2.871
SD	4.672	6.367	4.589	6.770	11.653	14.123	16.012	21.221	20.288	14.067

Table B.17 Raw data of the plating efficiency of HAN-treated CHO cells. The blank-corrected absorbency values were normalized to the concurrent negative control (%).

	Negative control	CAN			BAN			IAN		
		460 µM	790 µM	1200 µM	43 µM	52 µM	60 µM	45 µM	53 µM	60 µM
	105.440	88.978	82.923	70.057	47.351	30.889	16.698	16.698	4.778	3.453
	96.925	81.599	82.356	67.029	44.702	29.565	14.995	13.860	-0.142	-3.169
	97.871	87.654	80.274	73.463	46.405	31.079	18.212	13.103	1.750	-3.169
	98.439	90.303	87.465	72.706	47.919	29.754	18.590	14.806	-0.520	-1.466
	100.142	90.303	82.545	69.678	50.568	35.809	20.861	17.833	-0.142	-3.359
	93.898	85.383	72.517	64.948	49.432	29.186	13.482	13.103	-0.520	-4.872
	108.278	101.466	91.060	79.518	59.082	39.593	21.807	20.104	8.940	1.939
	99.007	88.600	85.951	76.490	61.731	34.295	22.185	18.023	5.913	1.372
	108.768	84.926	75.862	58.916	40.394	25.813	12.414	16.355	0.197	4.532
	95.961	82.759	75.862	52.020	35.862	20.296	8.670	13.005	-3.547	1.379
	97.931	88.670	77.438	60.690	34.877	25.419	12.414	10.640	-2.562	-2.365
	95.961	84.138	81.576	64.433	30.542	26.798	14.975	13.005	-3.547	-5.714
	99.901	87.291	85.123	67.586	33.103	29.754	19.113	12.020	-1.576	-1.379
	95.567	84.926	71.724	51.626	29.163	26.010	8.670	8.473	-4.729	-3.350
	106.010	99.901	94.581	64.631	38.818	34.286	17.537	14.384	2.365	-1.182
	99.901	87.882	83.941	56.946	30.739	33.695	13.399	13.596	0.394	-5.517
	98.888	87.720	71.381	61.246	39.736	24.224	10.367	6.644	-1.008	-1.629
	99.509	82.135	74.069	54.628	30.636	30.016	8.919	8.506	3.542	0.853
	97.441	74.690	76.758	58.557	34.566	28.154	10.160	9.540	1.267	-0.595
	101.991	77.792	75.517	61.867	35.393	30.636	13.883	9.747	0.646	0.026
	98.268	86.479	78.826	64.142	38.495	33.118	15.538	8.713	-0.595	-0.801
	86.065	76.758	68.485	65.589	32.704	28.775	4.576	7.472	-2.663	-3.490
	113.987	91.856	86.686	60.419	43.666	35.807	16.779	7.058	3.128	3.956
	103.852	77.999	77.172	67.244	42.632	31.050	15.538	6.644	-1.008	-0.595
number	24	24	24	24	24	24	24	24	24	24
Average	100.000	86.259	80.004	64.351	40.772	30.167	14.574	12.222	0.432	-1.048
SE	1.159	1.299	1.334	1.468	1.807	0.873	0.924	0.797	0.650	0.582
SD	5.680	6.365	6.538	7.194	8.852	4.276	4.527	3.904	3.183	2.849

Table B.18 Pearson correlation analysis of physicochemical and toxicological measurements of monohaloacetonitriles.

Pearson Product Moment Correlation Wednesday, August 21, 2013, 12:08:31 PM

Data source: Data 2 in Yukako HAN 2ANOVA

Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	Length (A)	**log P**	**EHOMO**	**ELUMO**	**t50 (min)**	**60% SCGE µM**
C-X	-- -- 0	-- -- 0	-- -- 0	-- -- 0	-- -- 0	-- -- 0
Length (A)		0.987 0.104 3	0.992 0.0803 3	-0.990 0.0908 3	0.616 0.578 3	-0.792 0.418 3
log P			0.958 0.184 3	-1.000 0.0131 3	0.480 0.681 3	-0.682 0.522 3
EHOMO				-0.964 0.171 3	0.710 0.497 3	-0.862 0.338 3
ELUMO					-0.498 0.668 3	0.697 0.509 3
t50 (min)						-0.969 0.159 3
60% SCGE µM						

The pair(s) of variables with positive correlation coefficients and P values below 0.050 tend to increase together. For the pairs with negative correlation coefficients and P values below 0.050, one variable tends to decrease while the other increases. For pairs with P values greater than 0.050, there is no significant relationship between the two variables.

APPENDIX C

APPENDICES FOR CHAPTER 4

Summary of contents:

- Ninety one pages.
- Appendix C.1 Cell cycle profiles of CHO cells treated without or with 100 µM of AraC with 0, 8, 14, 20, and 26 hour post-incubation.
- Appendix C.2 Cell cycle profiles of CHO cells treated with various concentrations of HANs at 26 hour post-incubation.
- Appendix C.3 Cell cycle profiles of CHO cells treated with different initial cell loading.
- Appendix C.4 Cell cycle profiles of CHO cells at 0, 8, 17, 26, 34, 43, and 52 hour post-incubation.
- Appendix C.5 CHO cell viability and cell numbers during post-incubation after treated without or with 900 µM CAN, 12 µM BAN, 6 µM IAN, 50 µM AraC, or 10 mM EMS.
- Appendix C.6 Cell cycle profiles of CHO cells treated without or with 10 mM EMS for 4 hours and harvested at 0, 26, and 52 hour post-incubation.
- Appendix C.7 Plating efficiency of HAN-treated CHO cells.
- Appendix C.8 Raw data of the DNA strand breaks measured by single cell gel electrophoresis assay.

Appendix C.1 Cell cycle profiles of CHO cells treated without or with 100 µM of AraC with 0, 8, 14, 20, and 26 hour post-incubation. DNA contents in individual cells were measured with LSR II flow cytometer and the cell cycle distributions were calculated using the FCS Express software. A summary table, cell cycle distribution, and fitted curves as well as the fitting models are provided (raw data used for Figure 4.3).

Table C.1 Summary of cell cycle distribution (%) of the different treatment groups.

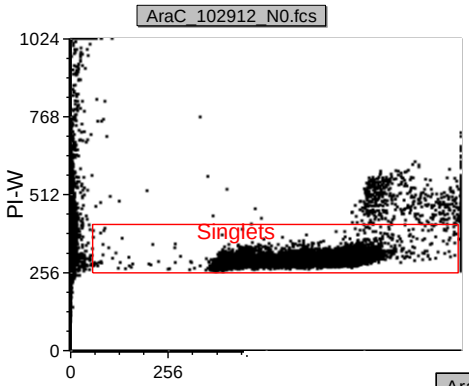
Negative control

Time (hours)	2N	2N to 4N	4N
0	37.57	43.47	18.96
8	30.01	62.17	7.81
14	15.56	53.78	30.67
20	35.66	51.04	13.30
26	31.73	53.56	14.71

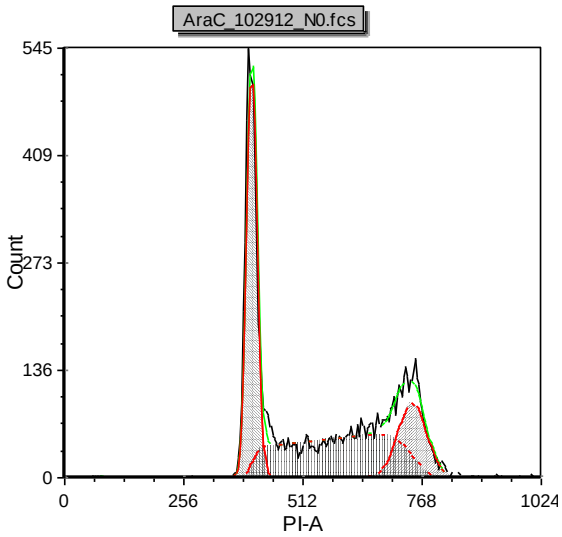
AraC 100 µM

Time (hours)	2N	2N to 4N	4N
0	26.35	54.72	18.92
8	4.02	68.79	27.19
14	4.07	5.85	90.09
20	6.96	33.4	59.65
26	14.13	7.53	78.35

Negative control, 0 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL S0	21.97	41.04	1.64
SL CL S0	20.96	41.88	1.44
+G2/G1 Fixed	1.11	59.85	4.98
+Aggregates	0.79	59.80	5.19
+S Order = 1	18.96	43.47	1.55
+CVs Fixed	14.47	48.23	1.94



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	403.93	2.85	37.57	749.27	4.15	18.96	43.47	1.86	100.00	0.21

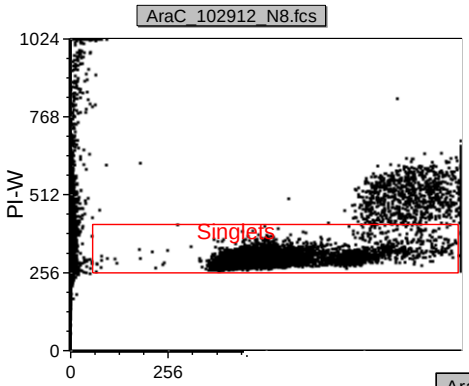
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=43.5, %G2=19.
The S Phase confidence is fair
Note: inter-model error,

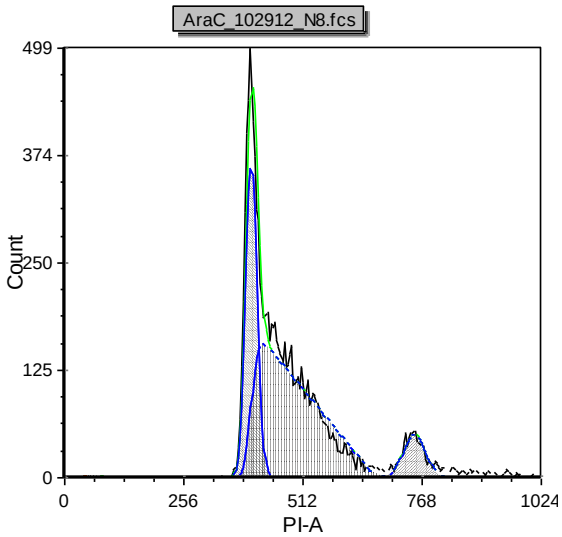
Experiment Statistics

Chi sq: 1.55
BAD: 0.21
Number of cells: 10084.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Negative control, 8 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV S1	100.00	0.00	19.54	35.17	22.42
SL CL S0	71.36	3.45	0.00	28.62	14.81
+G2/G1 Fixed	0.00	0.00	1.90	26.02	14.71
+Aggregates	75.13	24.87	0.00	28.05	14.70
+S Order = 1	0.00	100.00	7.81	62.17	2.66
+CVs Fixed	82.95	0.00	0.00	0.00	45.44



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	37.91	29.64	0.00	80.95	29.64	0.00	100.00	2.14	0.20	n/a	64.58
Aneuploid A	402.58	3.13	30.01	753.91	3.13	7.81	62.17	1.87	99.80	10.62	0.10

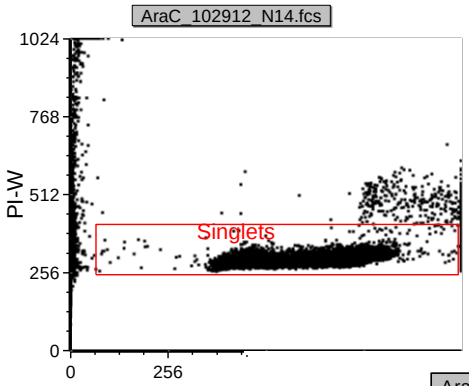
Interpretation

MultiCycle suggestions (a guideline only):
An aneuploid DNA content is observed.
The average %S=62.2, %G2=7.8
The S Phase confidence is poor
Note: intra-model error, inter-model error,

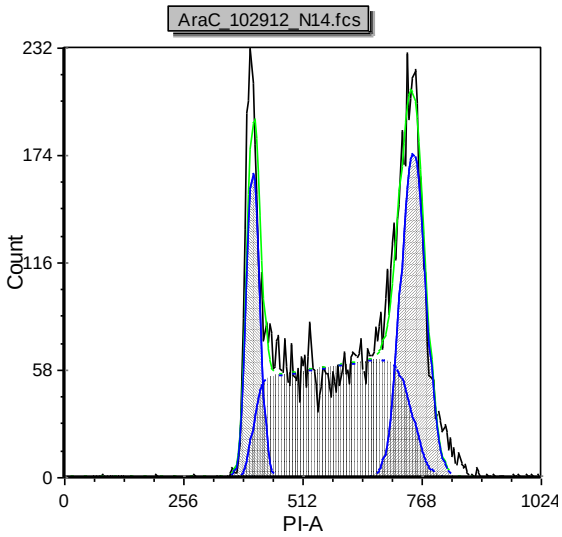
Experiment Statistics

Chi sq: 2.66
Average %S: 62.25
Average %S aneuploid: 62.17
BAD: 0.34
Number of cells: 10094.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

Negative control, 14 hr, +S order= 1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV S0	0.00	0.00	31.79	53.05	2.26
SL CL S0	0.00	100.00	31.51	53.41	2.06
+G2/G1 Fixed	8.71	5.99	30.55	54.41	2.14
+Aggregates	0.00	0.00	31.69	53.23	2.04
+S Order = 1	9.24	58.60	30.67	53.78	2.25
+CVs Fixed	33.33	66.67	0.00	11.80	56.40



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	292.31	380.78	32.16	535.69	33.00	9.24	58.60	1.83	4.26	n/a	0.70
Aneuploid A	406.18	3.57	15.56	751.53	3.57	30.67	53.78	1.85	95.74	1.39	0.37

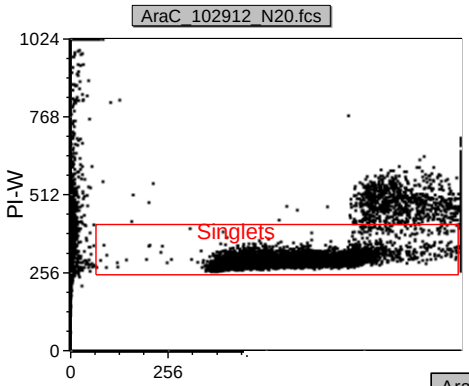
Interpretation

MultiCycle suggestions (a guideline only):
An aneuploid DNA content is observed.
The aneuploid %S=53.8, %G2=30.7
The S Phase confidence is fair
Note: inter-model error,

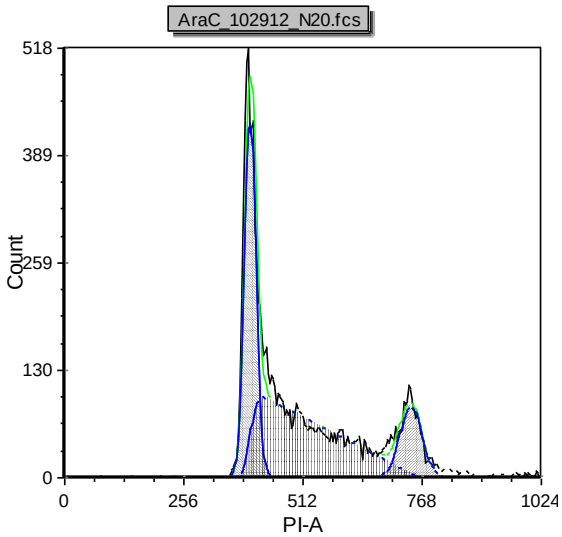
Experiment Statistics

Chi sq: 2.25
Average %S: 53.98
Average %S aneuploid: 53.78
BAD: 0.48
Number of cells: 10052.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

Negative control, 20 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV S0	19.93	0.00	8.29	49.01	4.66
SL CL S0	0.00	38.62	8.50	48.86	3.94
+G2/G1 Fixed	12.15	0.00	0.00	54.85	7.58
+Aggregates	0.00	0.00	8.01	49.35	3.91
+S Order = 1	0.00	73.15	13.30	51.04	2.24
+CVs Fixed	0.00	97.75	0.00	65.53	4.85



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	86.22	15.24	26.85	161.24	6.53	0.00	73.15	1.87	0.04	n/a	58.32
Aneuploid A	400.05	3.17	35.66	746.56	3.17	13.30	51.04	1.87	99.96	4.64	0.10

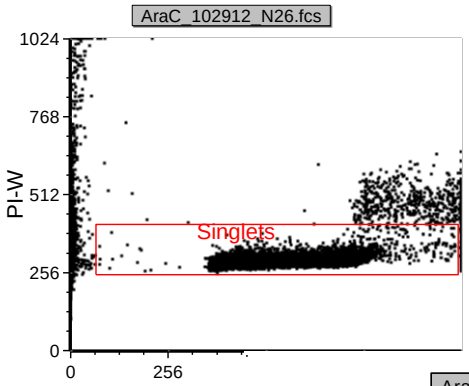
Interpretation

MultiCycle suggestions (a guideline only):
An aneuploid DNA content is observed.
The aneuploid %S=51., %G2=13.3
The S Phase confidence is good

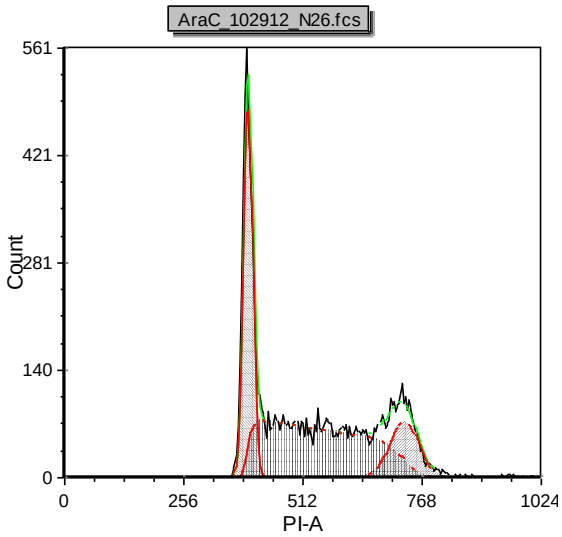
Experiment Statistics

Chi sq: 2.24
Average %S: 51.05
Average %S aneuploid: 51.04
BAD: 0.27
Number of cells: 10104.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

Negative control, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL S0	11.70	55.48	1.56
SL CL S0	11.08	56.01	1.08
+G2/G1 Fixed	0.00	67.30	2.48
+Aggregates	0.00	67.10	2.36
+S Order = 1	14.71	53.56	1.37
+CVs Fixed	9.58	57.64	1.84



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	395.66	2.56	31.73	732.47	4.29	14.71	53.56	1.85	100.00	0.08

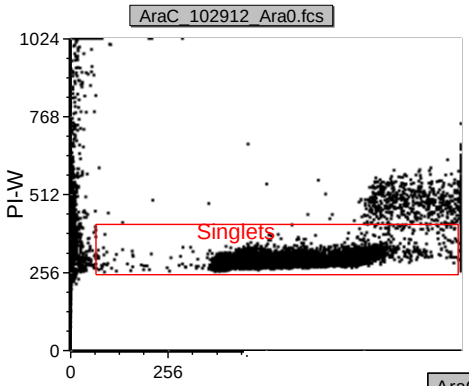
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=53.6, %G2=14.7
The S Phase confidence is good

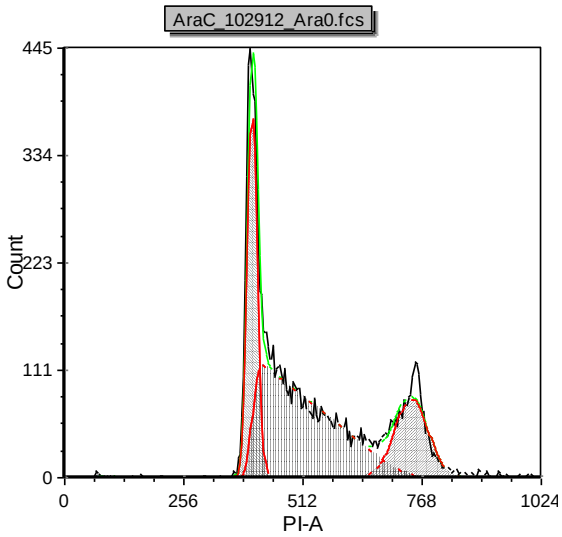
Experiment Statistics

Chi sq: 1.37
BAD: 0.08
Number of cells: 10071.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

AraC 100µM, 0 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	11.90	52.99	5.21
SL CL S0	9.36	56.18	3.89
+G2/G1 Fixed	11.97	52.90	5.21
+Aggregates	11.77	53.00	4.74
+S Order = 1	18.92	54.72	1.53
+CVs Fixed	14.27	57.26	2.09



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	404.79	2.70	26.35	747.36	4.78	18.92	54.72	1.85	100.00	0.29

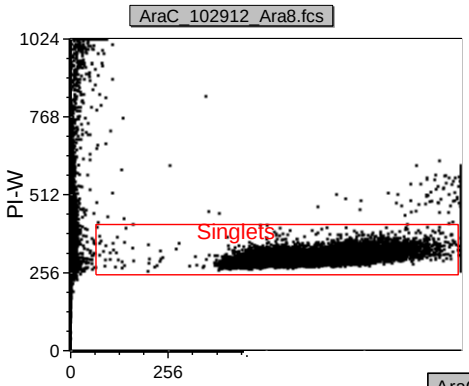
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=54.7, %G2=18.9
The S Phase confidence is good

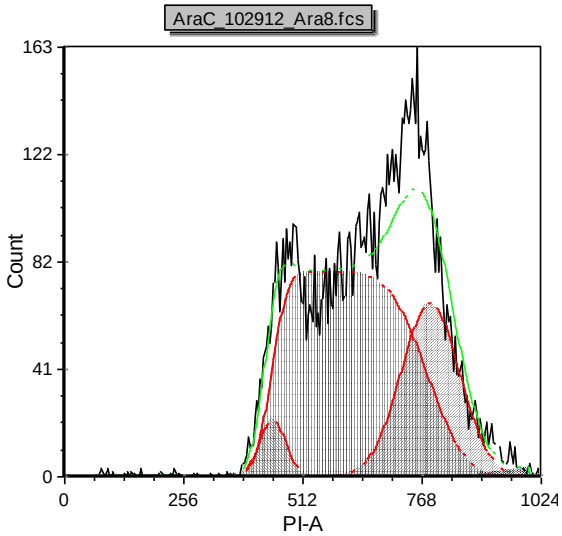
Experiment Statistics

Chi sq: 1.53
BAD: 0.29
Number of cells: 10105.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

AraC 100µM, 8 hr, +aggregates model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	5.67	90.14	5.94
SL CL S0	36.06	59.62	1.41
+G2/G1 Fixed	28.51	67.30	2.11
+Aggregates	27.19	68.79	1.91
+S Order = 1	25.07	67.49	2.12
+CVs Fixed	23.55	70.26	2.68



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	448.00	6.26	4.02	788.03	8.00	27.19	68.79	1.76	100.00	0.19

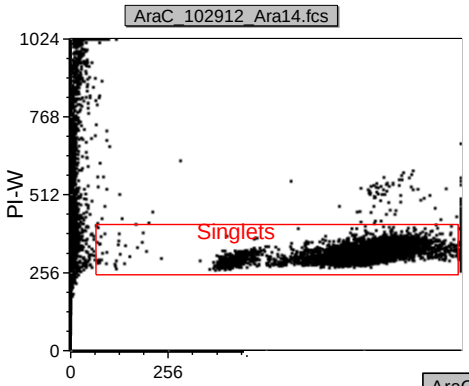
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=68.8, %G2=27.2
The S Phase confidence is fair
Note: inter-model error,

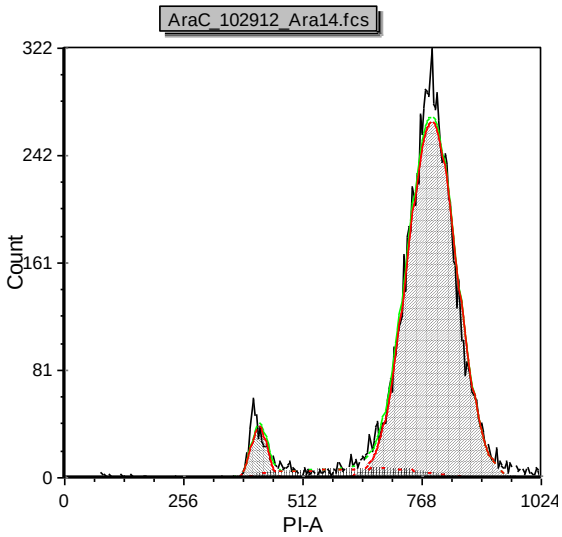
Experiment Statistics

Chi sq: 1.91
BAD: 0.19
Mean Xtra S: 659.37
Number of cells: 10100.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

AraC 100µM, 14 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	94.63	2.28	4.83
SL CL S0	91.80	4.03	1.36
+G2/G1 Fixed	92.62	3.03	3.64
+Aggregates	92.13	3.22	2.44
+S Order = 1	90.09	5.85	1.81
+CVs Fixed	84.04	12.16	6.93



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	418.54	3.94	4.07	791.64	6.73	90.09	5.85	1.89	100.00	0.46

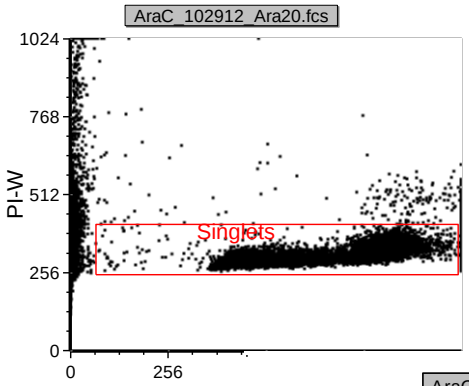
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=5.84, %G2=90.1
The S Phase confidence is fair
Note: inter-model error,

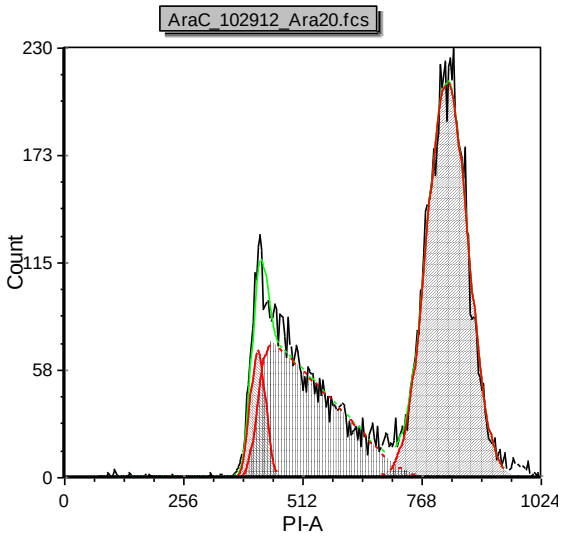
Experiment Statistics

Chi sq: 1.81
BAD: 0.46
Mean Xtra S: 659.37
Number of cells: 10133.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

AraC 100µM, 20 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	29.70	64.64	24.47
SL CL S0	53.62	26.02	2.65
+G2/G1 Fixed	54.49	25.10	2.97
+Aggregates	53.83	25.64	2.65
+S Order = 1	59.65	33.40	1.15
+CVs Fixed	59.41	30.91	1.29



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	416.64	3.87	6.96	825.10	5.38	59.65	33.40	1.98	100.00	0.90

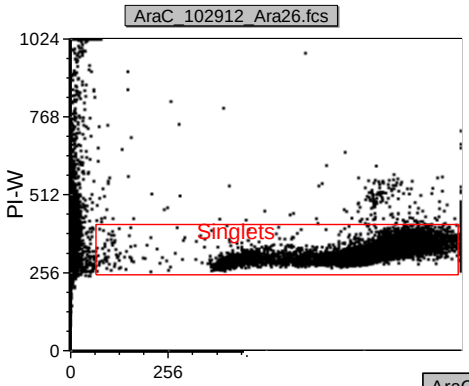
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=33.4, %G2=59.6
The S Phase confidence is fair
Note: inter-model error,

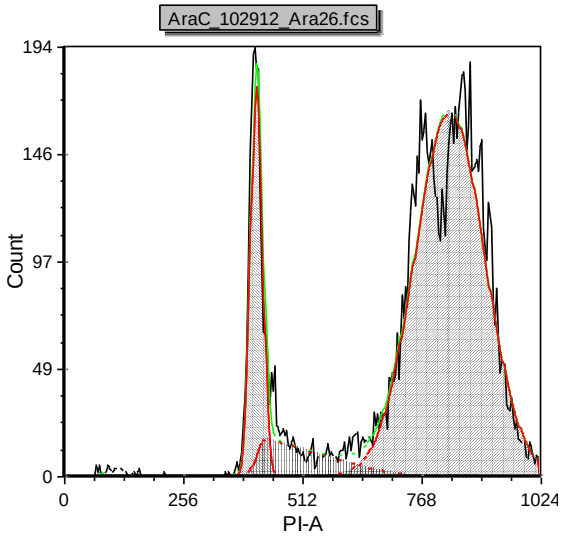
Experiment Statistics

Chi sq: 1.15
BAD: 0.90
Mean Xtra S: 659.37
Number of cells: 10283.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

AraC 100µM, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL S0	74.13	11.03	2.42
SL CL S0	76.57	10.95	4.54
+G2/G1 Fixed	76.35	8.02	3.76
+Aggregates	75.00	8.38	3.70
+S Order = 1	78.35	7.53	2.29
+CVs Fixed	75.90	13.80	6.53



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	413.84	3.07	14.13	830.37	9.07	78.35	7.53	2.01	100.00	1.33

Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=7.53, %G2=78.3
The S Phase confidence is fair
Note: inter-model error,

Experiment Statistics

Chi sq: 2.29
BAD: 1.33
Mean Xtra S: 659.37
Number of cells: 10518.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Appendix C.2 Cell cycle profiles of CHO cells treated with various concentrations of HANs at 26 hour post-incubation. DNA contents in individual cells were measured with LSR II flow cytometer and the cell cycle distributions were calculated using the FCS Express software. A summary table, cell cycle distribution, and fitted curves as well as the fitting models are provided (raw data used for Figure 4.4).

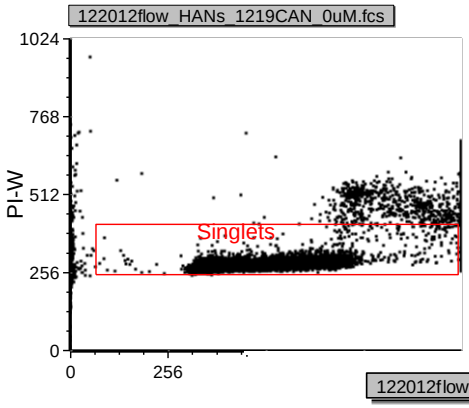
Table C.2 Summary of cell cycle distribution (%) of the different treatment groups.

CAN Concentration (μM)	2N	2N to 4N	4N	4N to 8N	8N
0	48.11	45.13	6.76	0	0
700	7.85	58.91	33.23	0	0
750	12.38	51.26	36.36	0	0
800	8.40	37.02	45.62	8.97	0
850	16.29	18.72	55.44	9.55	0
900	26.70	15.13	58.17	0	0

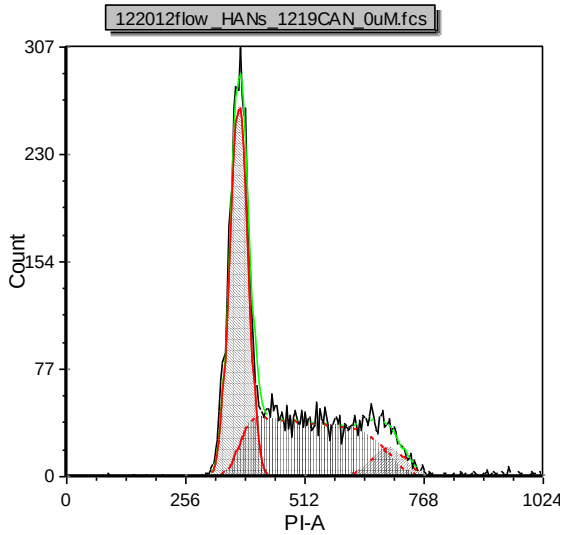
BAN Concentration (μM)	2N	2N to 4N	4N	4N to 8N	8N
0	44.01	47.09	8.90	0	0
9	52.78	30.95	16.28	0	0
10	21.34	20.64	36.97	21.05	0
11	11.30	26.28	33.08	29.35	0
12	2.08	18.17	46.27	33.50	0
13	0.68	29.35	29.63	40.34	0

IAN Concentration (μM)	2N	2N to 4N	4N	4N to 8N	8N
0	49.17	42.41	8.42	0	0
2	41.21	46.96	11.84	0	0
3	30.89	51.82	17.29	0	0
4	31.24	43.36	25.40	0	0
6	1.58	5.78	56.81	33.68	2.15
8	0.60	2.38	69.67	25.19	2.16

CAN 0 µM, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL S0	2.73	46.85	2.08
SL CL S0	0.00	50.39	1.17
+G2/G1 Fixed	0.00	52.07	4.04
+Aggregates	0.00	53.94	4.26
+S Order = 1	6.76	45.13	1.33
+CVs Fixed	6.87	45.03	1.32



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	371.44	5.34	48.11	694.74	5.28	6.76	45.13	1.87	100.00	0.22

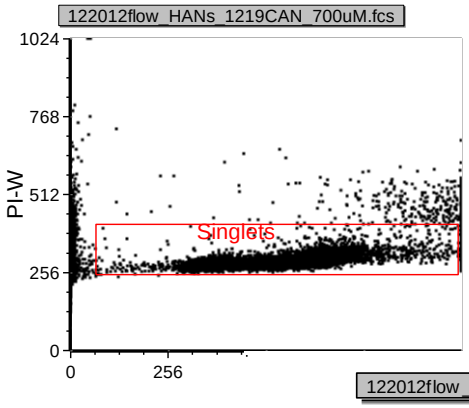
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=45.1, %G2=6.76
The S Phase confidence is good

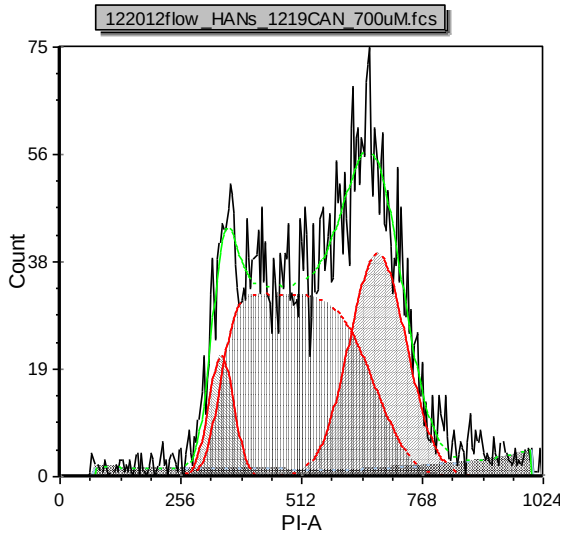
Experiment Statistics

Chi sq: 1.33
BAD: 0.22
Mean Xtra S: 659.37
Number of cells: 7140.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

CAN 700 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	46.95	41.54	1.88
SL CL S0	33.23	58.91	1.22
+G2/G1 Fixed	47.00	41.52	1.88
+Aggregates	45.05	43.54	1.46
+S Order = 1	44.33	50.60	1.73
+CVs Fixed	35.44	54.57	1.80



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	342.51	7.72	7.85	675.39	8.99	33.23	58.91	1.97	100.00	3.12

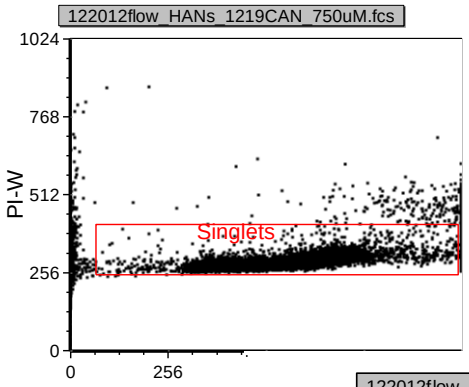
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=58.9, %G2=33.2
The S Phase confidence is good

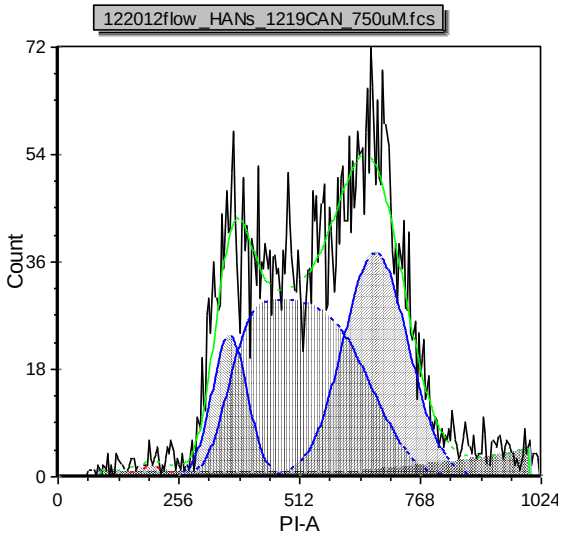
Experiment Statistics

Chi sq: 1.22
BAD: 3.12
Mean Xtra S: 659.37
Number of cells: 5125.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

CAN 750 µM, 26 hr, +aggregates model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S0	0.00	6.77	38.45	48.65	2.07
SL CL S0	3.34	95.46	30.44	61.30	1.40
+G2/G1 Fixed	0.00	0.00	38.29	48.97	2.05
+Aggregates	0.00	0.00	36.36	51.26	1.48
+S Order = 1	0.00	0.00	36.42	51.26	2.10
+CVs Fixed	0.00	0.00	39.31	60.69	1391135.00



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	201.97	18.11	100.00	373.65	18.21	0.00	0.00	1.85	0.83	n/a	6.29
Aneuploid A	364.58	10.20	12.38	674.72	10.20	36.36	51.26	1.85	99.17	1.81	2.11

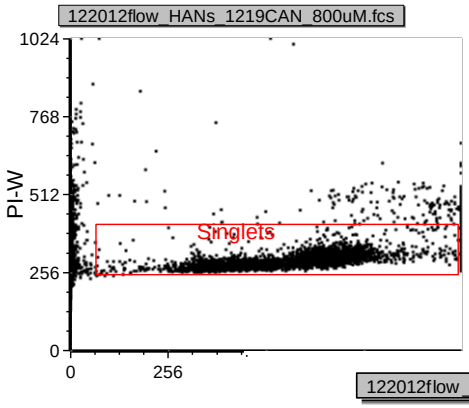
Interpretation

MultiCycle suggestions (a guideline only):
An aneuploid DNA content is observed.
The average %S=50.8, %G2=36.1
The S Phase confidence is good

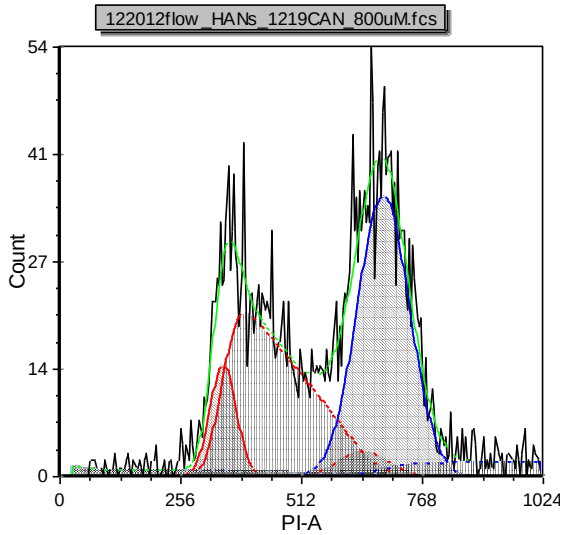
Experiment Statistics

Chi sq: 1.48
Average %S: 50.83
Average %S aneuploid: 51.26
BAD: 2.81
Mean Xtra S: 659.37
Number of cells: 5128.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

CAN 800 µM, 26 hr, +CVs fixed model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	0.00	72.06	0.00	17.67	1.02
SL CL S0	0.00	69.42	0.00	13.42	1.03
+G2/G1 Fixed	0.00	69.73	0.00	18.39	1.01
+Aggregates	0.00	69.99	0.00	14.31	1.07
+S Order = 1	0.00	70.09	0.00	17.74	1.06
+CVs Fixed	7.35	75.51	0.00	17.60	0.95



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	346.17	8.03	17.14	645.91	8.11	7.35	75.51	1.87	49.03	n/a	2.73
Aneuploid A	687.45	8.06	82.41	1273.88	8.06	0.00	17.60	1.85	50.98	1.99	0.77

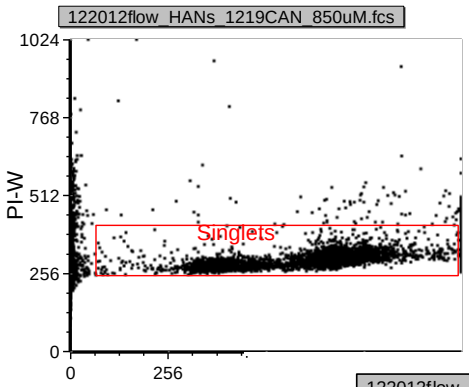
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=17.6, %G2=0.
The S Phase confidence is poor
Note: intra-model error,

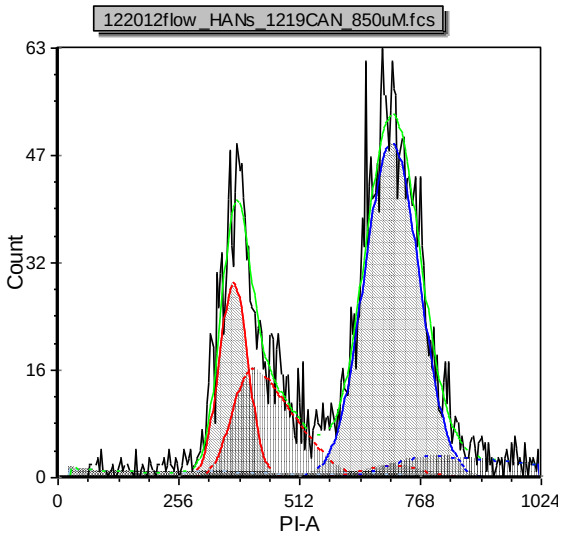
Experiment Statistics

Chi sq: 0.95
Average %S: 45.99
Average %S aneuploid: 17.60
BAD: 1.91
Mean Xtra S: 659.37
Number of cells: 3094.00
Number of cycles: 2.00

CAN 850 µM, 26 hr, +CVs fixed model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	0.00	48.12	0.00	14.25	1.12
SL CL S0	0.00	39.76	0.00	9.50	1.34
+G2/G1 Fixed	0.00	40.63	0.00	19.96	1.26
+Aggregates	0.00	40.08	0.00	10.68	1.30
+S Order = 1	0.00	40.84	0.00	18.69	1.27
+CVs Fixed	5.43	50.57	0.00	15.16	1.12



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	373.10	8.20	44.00	713.55	8.24	5.43	50.57	1.91	37.02	n/a	2.57
Aneuploid A	709.59	8.22	84.84	1313.74	8.22	0.00	15.16	1.85	62.98	1.90	0.75

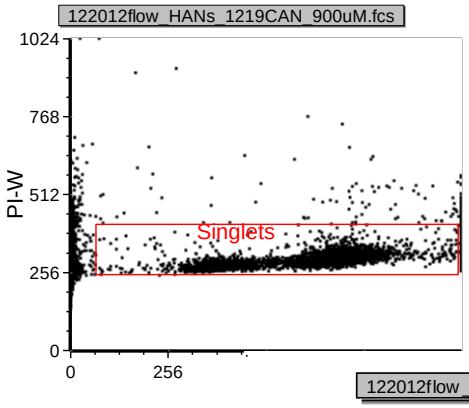
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=15.2, %G2=0.
The S Phase confidence is poor
Note: intra-model error, inter-model error,

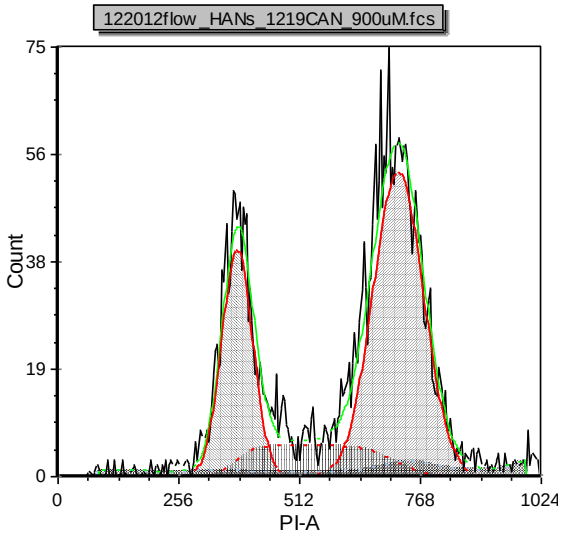
Experiment Statistics

Chi sq: 1.12
Average %S: 28.27
Average %S aneuploid: 15.16
BAD: 1.92
Mean Xtra S: 659.37
Number of cells: 3596.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

CAN 900 µM, 26 hr, +aggregates model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV S1	65.37	12.36	1.12
SL CL S0	58.93	13.99	0.96
+G2/G1 Fixed	59.58	14.37	1.17
+Aggregates	58.17	15.13	0.98
+S Order = 1	65.37	12.36	1.12
+CVs Fixed	65.23	12.28	1.11



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	380.89	8.67	26.70	723.29	7.39	58.17	15.13	1.90	100.00	4.92

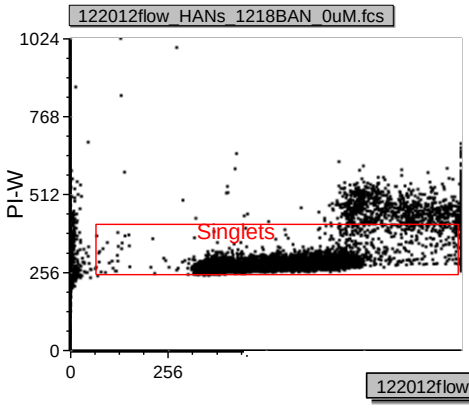
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=15.1, %G2=58.2
The S Phase confidence is good

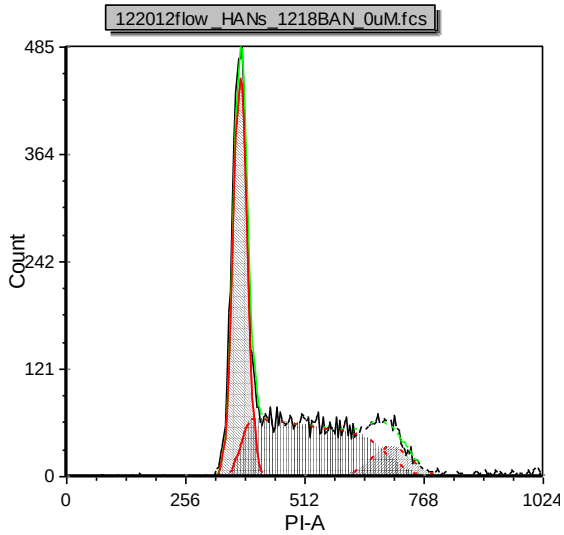
Experiment Statistics

Chi sq: 0.98
BAD: 4.92
Mean Xtra S: 659.37
Number of cells: 3568.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAN 0 µM, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	11.06	45.15	1.49
SL CL S0	4.32	49.62	0.99
+G2/G1 Fixed	8.90	45.92	1.63
+Aggregates	7.02	46.67	1.11
+S Order = 1	8.90	47.09	1.47
+CVs Fixed	5.00	50.30	1.57



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	374.16	4.09	44.01	700.70	5.84	8.90	47.09	1.87	100.00	0.24

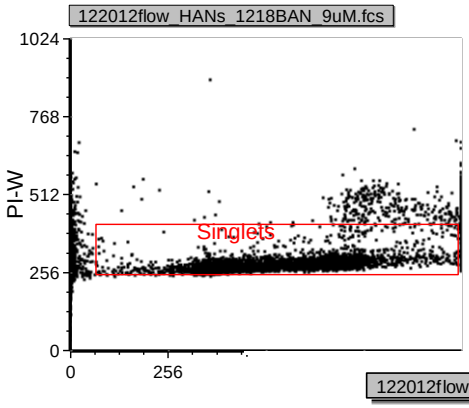
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=47.1, %G2=8.9
The S Phase confidence is good

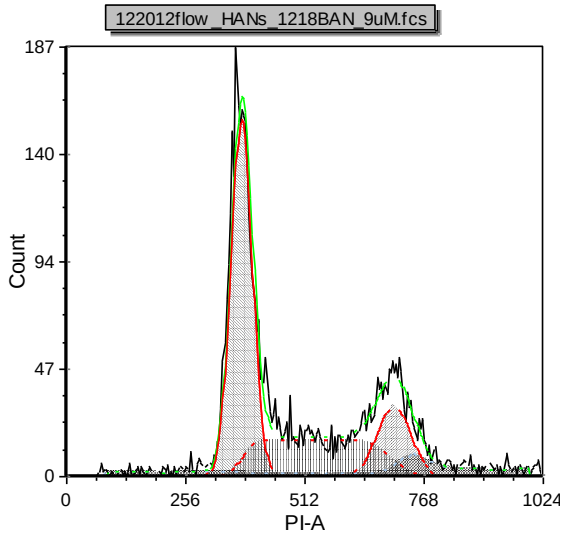
Experiment Statistics

Chi sq: 1.47
BAD: 0.24
Mean Xtra S: 659.37
Number of cells: 10187.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAN 9 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	31.58	25.28	1.66
SL CL S0	16.28	30.95	1.34
+G2/G1 Fixed	23.45	25.62	2.07
+Aggregates	16.87	29.60	1.40
+S Order = 1	29.15	26.33	1.63
+CVs Fixed	23.18	29.29	1.75



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	377.37	6.09	52.78	705.53	5.09	16.28	30.95	1.87	100.00	4.09

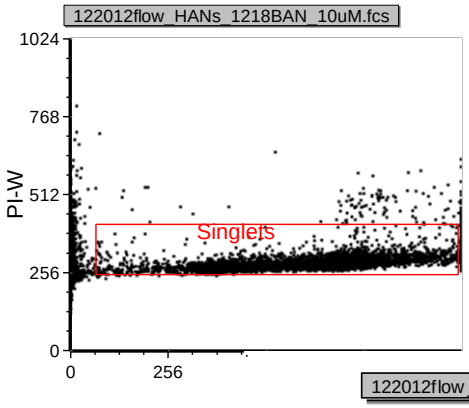
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=30.9, %G2=16.3
The S Phase confidence is good

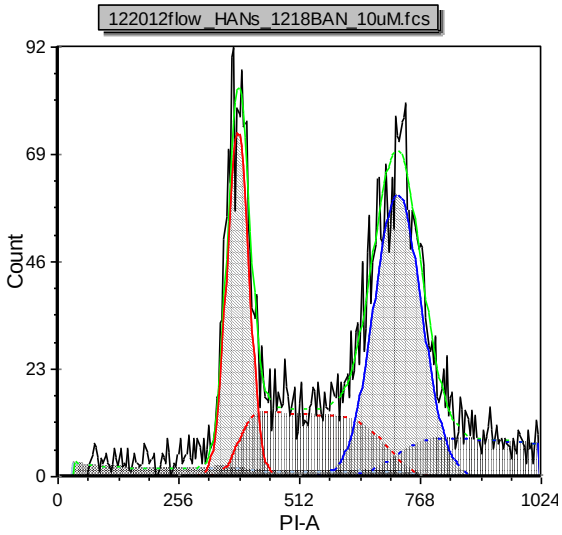
Experiment Statistics

Chi sq: 1.34
BAD: 4.09
Mean Xtra S: 659.37
Number of cells: 5133.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAN 10 µM, 26 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	0.00	49.22	0.00	38.51	1.55
SL CL S0	0.00	49.05	0.00	38.07	1.56
+G2/G1 Fixed	0.00	49.18	0.00	38.27	1.56
+Aggregates	0.00	48.88	0.00	36.24	1.55
+S Order = 1	0.00	49.17	0.00	36.28	1.57
+CVs Fixed	0.00	0.00	0.00	33.00	3.89



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	382.39	6.24	50.83	704.23	6.24	0.00	49.17	1.84	41.98	n/a	4.12
Aneuploid A	722.20	6.98	63.72	1332.49	6.98	0.00	36.28	1.85	58.02	1.89	1.24

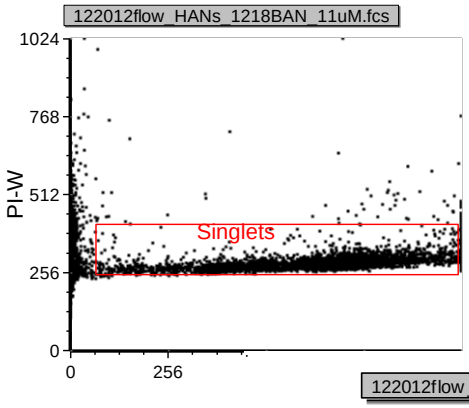
Interpretation

MultiCycle suggestions (a guideline only):
An aneuploid DNA content is observed.
The aneuploid %S=36.3, %G2=0.
The S Phase confidence is poor
Note: intra-model error,

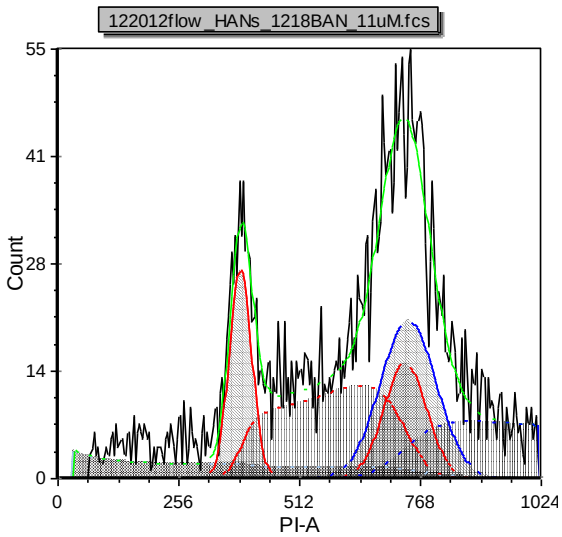
Experiment Statistics

Chi sq: 1.57
Average %S: 41.69
Average %S aneuploid: 36.28
BAD: 2.89
Mean Xtra S: 659.37
Number of cells: 5145.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

BAN 11 µM, 26 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	2.31	66.66	0.00	42.46	1.68
SL CL S0	5.36	64.46	0.00	17.96	1.75
+G2/G1 Fixed	0.00	68.31	0.00	45.29	1.77
+Aggregates	0.79	65.77	0.00	20.89	1.76
+S Order = 1	23.97	53.17	0.00	58.02	1.71
+CVs Fixed	0.35	67.96	0.00	39.89	1.69



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	388.11	6.15	22.86	738.19	6.15	23.97	53.17	1.90	49.42	n/a	7.01
Aneuploid A	744.71	7.96	41.98	1360.85	7.96	0.00	58.02	1.83	50.58	1.92	2.16

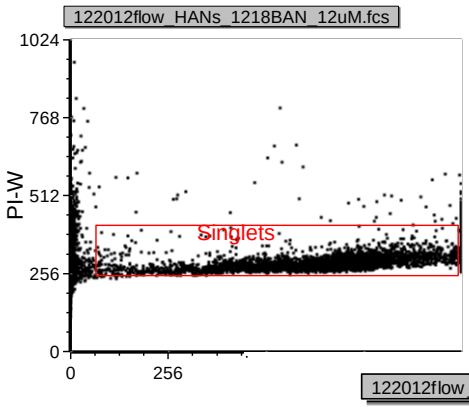
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=58., %G2=0.
The S Phase confidence is fair
Note: inter-model error,

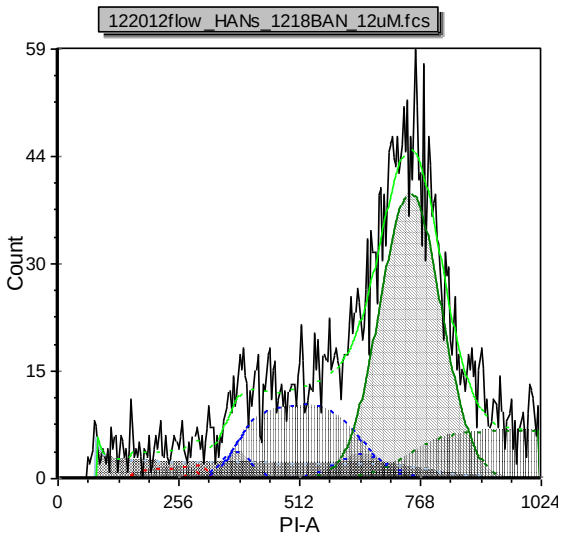
Experiment Statistics

Chi sq: 1.71
Average %S: 55.62
Average %S aneuploid: 58.02
BAD: 5.13
Mean Xtra S: 659.37
Number of cells: 3654.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

BAN 12 µM, 26 hr, +S order=1 model, 3 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	B %G2	B %S	Chis
SL CV G21 S1	35.93	56.34	9.57	82.19	0.00	44.38	1.21
SL CL S0	67.78	25.57	10.96	83.15	50.69	16.00	1.22
+G2/G1 Fixed	80.70	8.11	30.70	64.90	0.00	47.72	1.22
+Aggregates	67.44	32.37	6.91	90.05	0.00	41.19	1.46
+S Order = 1	25.71	69.38	13.50	77.64	0.00	43.73	1.23
+CVs Fixed	35.80	60.44	19.08	70.96	0.00	43.37	1.22



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	162.92	4.98	4.91	300.14	5.08	25.71	69.38	1.84	1.79	n/a	55.30
Aneuploid A	378.48	8.65	8.87	646.51	8.65	13.50	77.64	1.71	22.98	2.32	14.43
Aneuploid B	749.12	8.65	56.27	1486.90	8.65	0.00	43.73	1.99	75.24	4.60	3.00

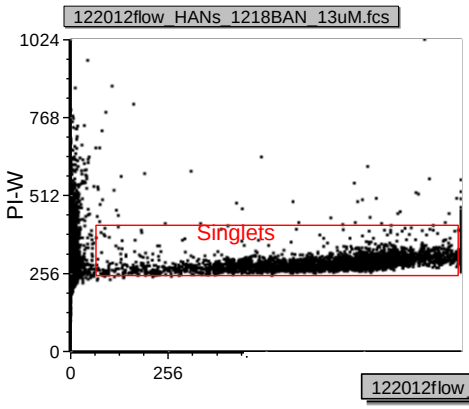
Interpretation

MultiCycle suggestions (a guideline only):
Multiple aneuploid DNA contents are observed.
The D.I. 2.32 aneuploid %S=77.6, %G2=13.5
The S Phase confidence is fair
Note: % cells, inter-model error,
The D.I. 4.6 aneuploid %S=43.7, %G2=0.
The S Phase confidence is fair
Note: inter-model error,

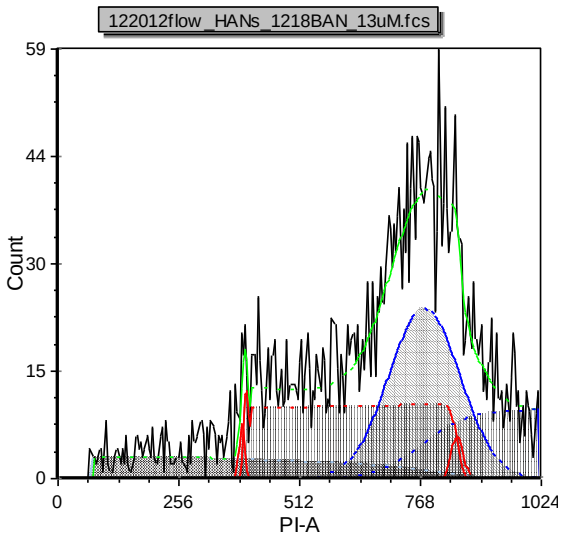
Experiment Statistics

Chi sq: 1.23
Average %S: 51.98
Average %S aneuploid: 51.66
BAD: 10.15
Mean Xtra S: 659.37
Number of cells: 3658.00
Number of cycles: 3.00
Cycle fit model: 3 Cycle

BAN 13 µM, 26 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	20.47	72.98	74.49	14.48	1.59
SL CL S0	3.20	94.37	0.00	57.21	1.43
+G2/G1 Fixed	0.32	96.16	92.04	3.87	1.46
+Aggregates	15.73	78.50	75.59	2.08	1.58
+S Order = 1	3.75	94.06	0.00	58.63	1.43
+CVs Fixed	0.00	0.00	96.80	0.00	2.39



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	390.72	1.43	2.19	848.58	1.45	3.75	94.06	2.17	31.20	n/a	8.37
Aneuploid A	778.65	9.70	41.37	1436.35	9.70	0.00	58.63	1.85	68.80	1.99	1.80

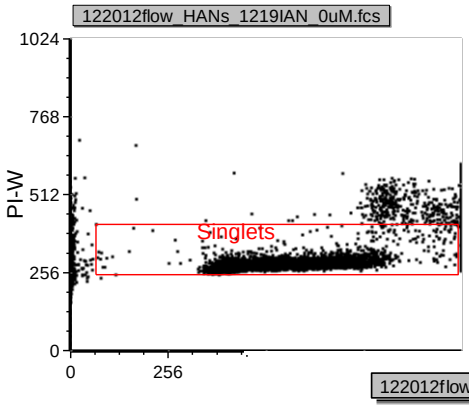
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=58.6, %G2=0.
The S Phase confidence is fair
Note: inter-model error,

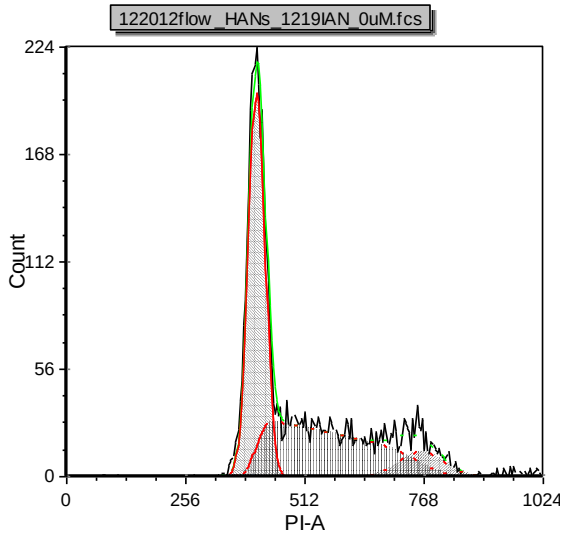
Experiment Statistics

Chi sq: 1.43
Average %S: 69.69
Average %S aneuploid: 58.63
BAD: 7.01
Mean Xtra S: 659.37
Number of cells: 3646.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

IAN 0 µM, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	10.29	40.83	0.99
SL CL S0	1.75	45.66	1.05
+G2/G1 Fixed	7.21	40.53	1.22
+Aggregates	4.50	42.46	1.16
+S Order = 1	8.42	42.41	0.98
+CVs Fixed	4.84	45.17	1.03



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	409.60	4.43	49.17	767.41	6.15	8.42	42.41	1.87	100.00	0.41

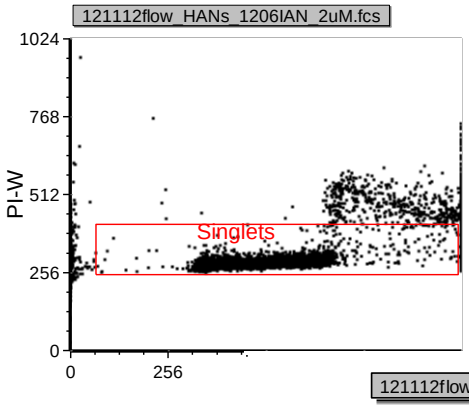
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=42.4, %G2=8.42
The S Phase confidence is poor
Note: intra-model error,

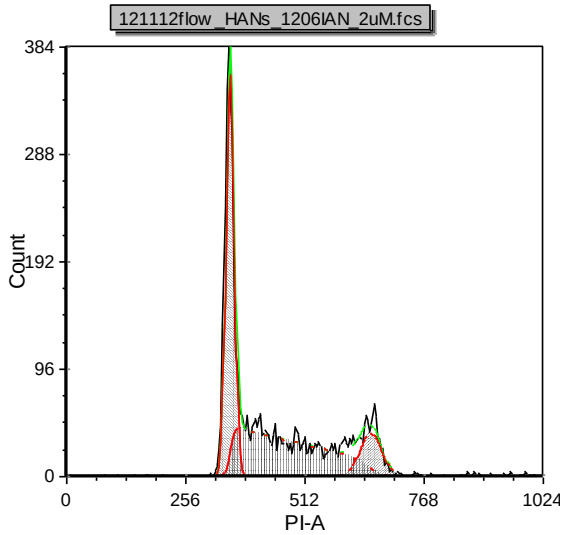
Experiment Statistics

Chi sq: 0.98
BAD: 0.41
Mean Xtra S: 659.37
Number of cells: 4853.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAN 2 µM, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	12.80	46.13	1.35
SL CL S0	6.98	49.30	1.37
+G2/G1 Fixed	9.88	45.96	1.89
+Aggregates	9.42	46.28	1.60
+S Order = 1	11.84	46.96	1.35
+CVs Fixed	9.29	48.91	1.42



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	352.54	2.49	41.21	655.36	3.54	11.84	46.96	1.86	100.00	0.44

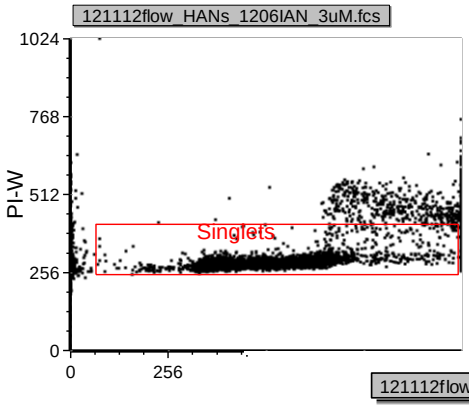
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=47., %G2=11.8
The S Phase confidence is good

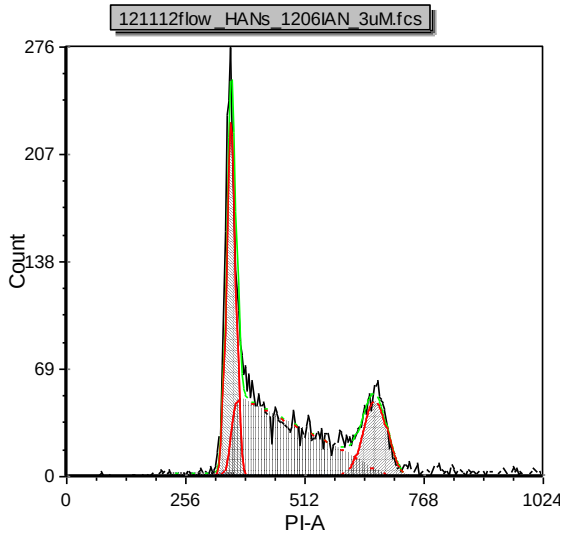
Experiment Statistics

Chi sq: 1.35
BAD: 0.44
Mean Xtra S: 659.37
Number of cells: 5091.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAN 3 µM, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	20.35	49.29	2.26
SL CL S0	10.05	53.47	2.17
+G2/G1 Fixed	13.62	49.20	3.56
+Aggregates	12.57	49.68	2.75
+S Order = 1	17.29	51.82	2.12
+CVs Fixed	14.61	53.25	2.23



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	354.43	2.77	30.89	664.86	3.94	17.29	51.82	1.88	100.00	1.93

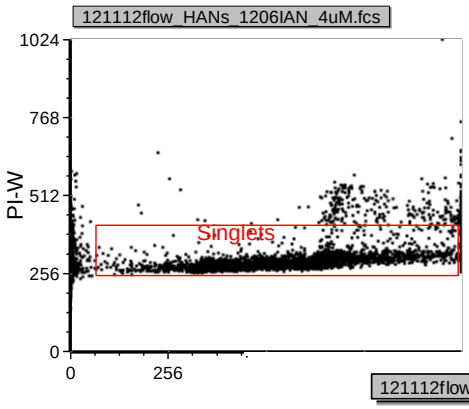
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=51.8, %G2=17.3
The S Phase confidence is poor
Note: intra-model error,

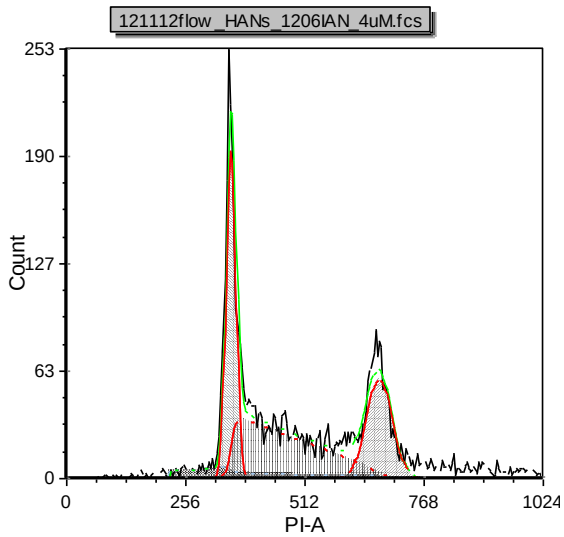
Experiment Statistics

Chi sq: 2.12
BAD: 1.93
Mean Xtra S: 659.37
Number of cells: 5122.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAN 4 µM, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	28.94	40.38	3.46
SL CL S0	17.39	45.88	2.13
+G2/G1 Fixed	21.61	42.40	4.26
+Aggregates	17.69	44.82	2.29
+S Order = 1	25.40	43.36	3.33
+CVs Fixed	22.91	44.66	3.44



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	354.38	2.92	31.24	673.80	4.10	25.40	43.36	1.90	100.00	6.63

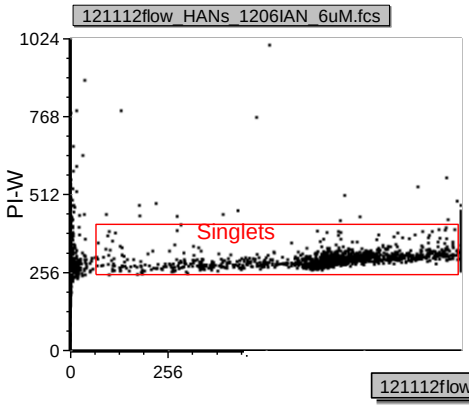
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=43.4, %G2=25.4
The S Phase confidence is poor
Note: intra-model error,

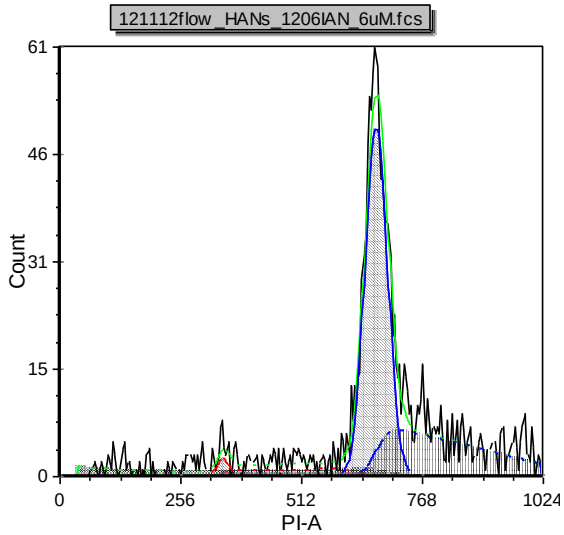
Experiment Statistics

Chi sq: 3.33
BAD: 6.63
Mean Xtra S: 659.37
Number of cells: 5122.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAN 6 µM, 26 hr, +CV fixed model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S0	0.00	80.12	2.13	41.61	1.23
SL CL S0	0.00	78.06	2.08	42.65	1.26
+G2/G1 Fixed	0.00	78.26	2.12	41.63	1.22
+Aggregates	0.00	78.11	2.08	42.59	1.24
+S Order = 1	0.00	77.81	2.13	41.20	1.24
+CVs Fixed	0.00	78.59	2.32	36.36	1.16



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	345.82	3.58	21.42	643.66	3.58	0.00	78.59	1.86	7.36	n/a	22.42
Aneuploid A	672.63	3.58	61.32	1255.41	3.58	2.32	36.36	1.87	92.64	1.95	2.23

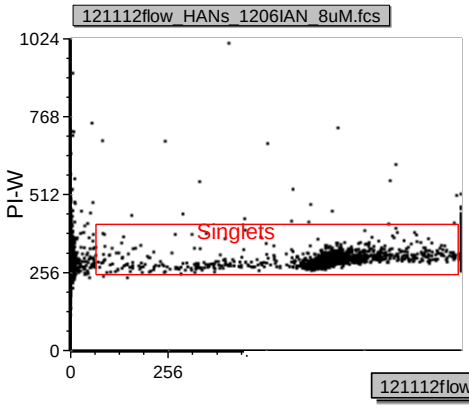
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=36.4, %G2=2.32
The S Phase confidence is good

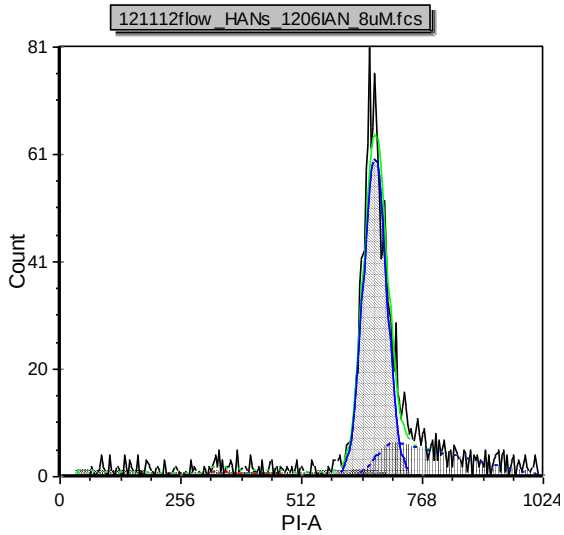
Experiment Statistics

Chi sq: 1.16
Average %S: 39.47
Average %S aneuploid: 36.36
BAD: 6.12
Mean Xtra S: 659.37
Number of cells: 1567.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

IAN 8 µM, 26 hr, +CVs fixed model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S0	0.00	77.10	2.19	27.49	1.14
SL CL S0	0.00	77.16	2.19	28.48	1.18
+G2/G1 Fixed	0.00	76.65	2.19	27.69	1.13
+Aggregates	0.00	77.03	2.20	28.25	1.16
+S Order = 1	0.00	76.02	2.21	26.92	1.15
+CVs Fixed	0.00	79.92	2.23	25.96	0.93



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	333.23	3.73	20.08	619.12	3.73	0.00	79.92	1.86	2.98	n/a	40.02
Aneuploid A	669.68	3.73	71.81	1248.16	3.73	2.23	25.96	1.86	97.02	2.01	1.66

Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=26., %G2=2.23
The S Phase confidence is good

Experiment Statistics

Chi sq: 0.93
Average %S: 27.57
Average %S aneuploid: 25.96
BAD: 5.53
Mean Xtra S: 659.37
Number of cells: 1580.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

Appendix C.3 Cell cycle profiles of CHO cells treated with different initial cell loading. Treatment conditions are negative control, 900 µM CAN, 12 µM BAN, and 6 µM IAN with 26 hour post-incubation. DNA contents in individual cells were measured with LSR II flow cytometer and the cell cycle distributions were calculated using the FCS Express software. A summary table, cell cycle distribution, and fitted curves as well as the fitting models are provided (raw data used for Figure 4.5).

Table C.3 Summary of cell cycle distribution (%) of the different treatment groups.

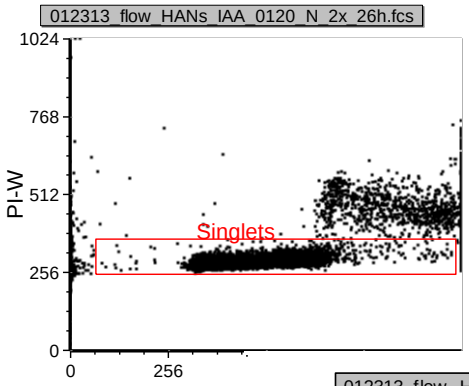
Initial loading: $2x10^5$ cells

Treatment group	2N	2N to 4N	4N	4N to 8N	8N
Negative	34.59	53.24	12.17	0	0
CAN 900 µM	18.22	17.73	64.05	0	0
BAN 12 µM	6.21	35.14	40.35	17.34	0.97
IAN 6 µM	1.09	9.44	49.18	39.78	0.51

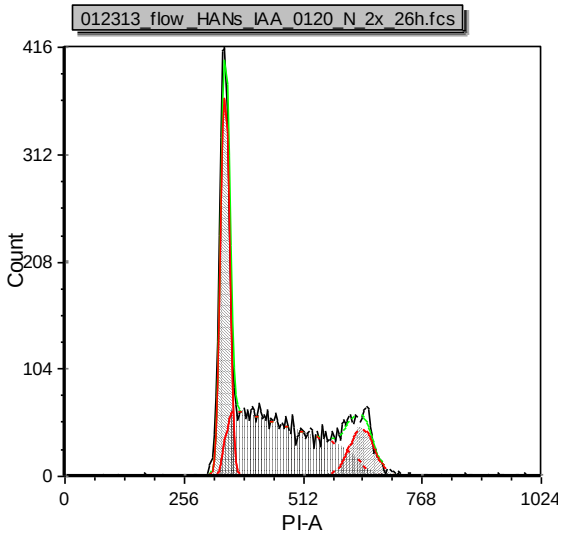
Initial loading: $4x10^5$ cells

Treatment group	2N	2N to 4N	4N	4N to 8N	8N
Negative	65.14	23.94	10.92	0	0
CAN 900 µM	20.85	21.12	58.03	0	0
BAN 12 µM	25.30	49.50	25.20	0	0
IAN 6 µM	32.32	28.04	18.52	20.43	0.69

Negative control, initial cell loading 2×10^5 cells/well, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	12.55	52.94	1.14
SL CL S0	5.78	56.77	1.35
+G2/G1 Fixed	9.32	53.25	1.73
+Aggregates	8.82	53.43	1.50
+S Order = 1	12.17	53.24	1.15
+CVs Fixed	8.52	56.12	1.26



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	345.12	2.79	34.59	639.57	4.12	12.17	53.24	1.85	100.00	0.34

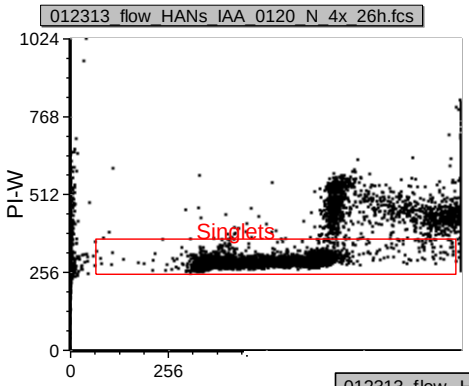
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=53.2, %G2=12.2
The S Phase confidence is poor
Note: intra-model error,

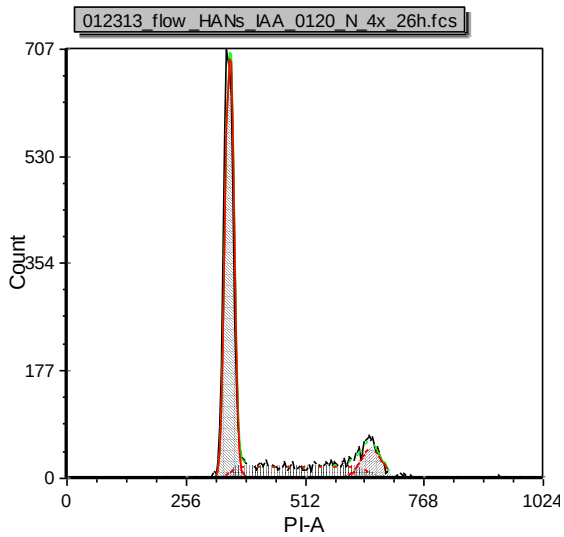
Experiment Statistics

Chi sq: 1.15
BAD: 0.34
Number of cells: 6652.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Negative control, initial cell loading 4×10^5 cells/well, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	12.38	22.40	1.35
SL CL S0	9.52	24.46	1.13
+G2/G1 Fixed	12.07	22.56	1.36
+Aggregates	10.04	23.66	1.19
+S Order = 1	10.92	23.94	1.27
+CVs Fixed	9.48	25.03	1.34



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	351.11	2.67	65.14	655.85	3.45	10.92	23.94	1.87	100.00	0.33

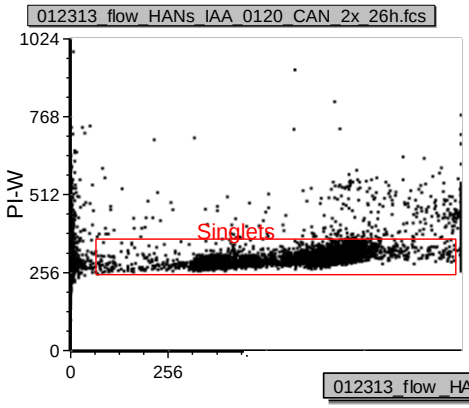
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=23.9, %G2=10.9
The S Phase confidence is good

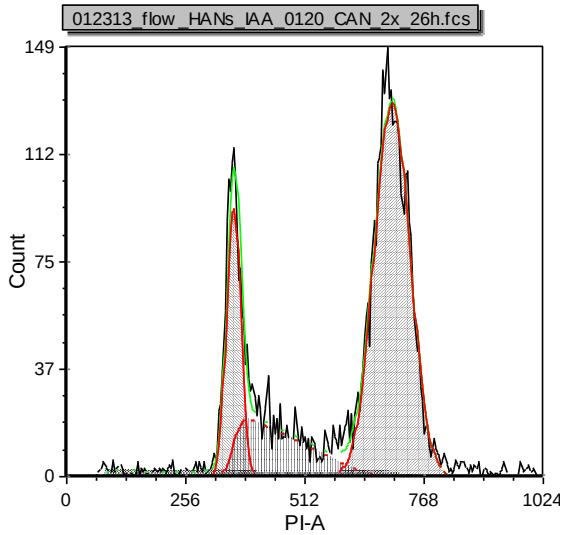
Experiment Statistics

Chi sq: 1.27
BAD: 0.33
Number of cells: 6639.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

CAN 900 µM, initial cell loading 2×10^5 cells/well, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S2	61.79	16.76	6.88
SL CL S0	59.25	19.25	1.57
+G2/G1 Fixed	57.40	16.12	5.68
+Aggregates	56.29	16.60	5.14
+S Order = 1	64.05	17.73	1.53
+CVs Fixed	62.73	17.65	1.67



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	360.19	4.22	18.22	701.73	5.48	64.05	17.73	1.95	100.00	3.97

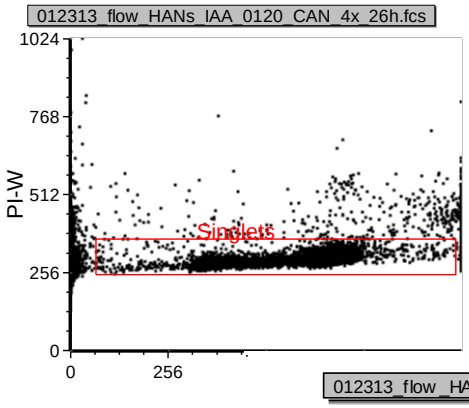
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=17.7, %G2=64.1
The S Phase confidence is good

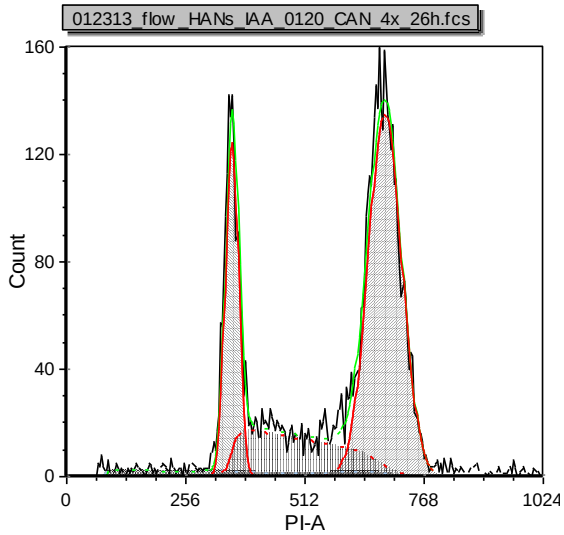
Experiment Statistics

Chi sq: 1.53
BAD: 3.97
Number of cells: 5488.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

CAN 900 µM, initial cell loading 4×10^5 cells/well, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S2	62.70	15.94	4.35
SL CL S0	55.71	22.38	1.25
+G2/G1 Fixed	56.74	18.45	3.96
+Aggregates	55.95	18.94	3.62
+S Order = 1	58.03	21.12	1.44
+CVs Fixed	55.80	22.30	1.67



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	356.53	3.96	20.85	686.17	5.28	58.03	21.12	1.93	100.00	3.72

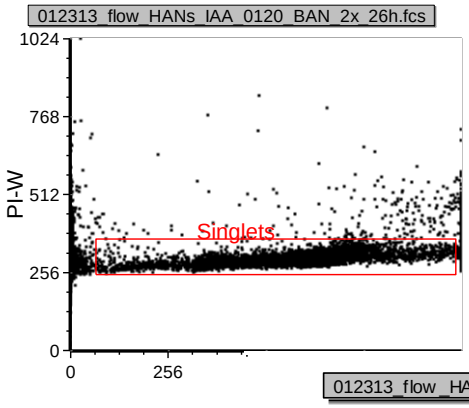
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=21.1, %G2=58.
The S Phase confidence is good

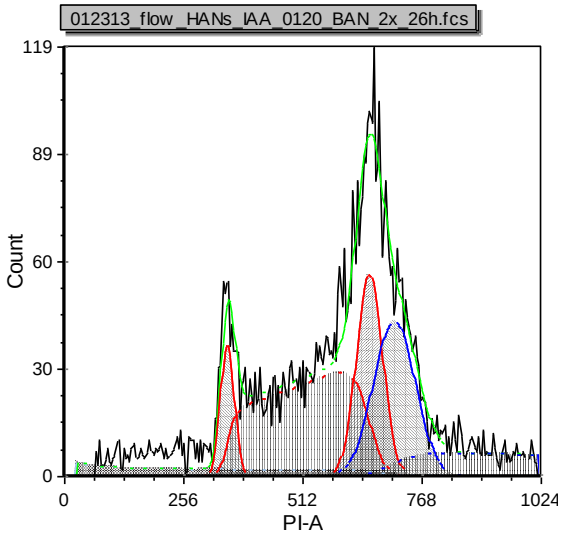
Experiment Statistics

Chi sq: 1.44
BAD: 3.72
Number of cells: 5885.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAN 12 µM, initial cell loading 2×10^5 cells/well, 26 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	31.37	59.34	2.38	42.54	2.68
SL CL S0	17.05	70.80	0.00	31.52	2.26
+G2/G1 Fixed	12.93	73.95	0.00	31.15	2.34
+Aggregates	11.52	74.99	0.00	33.02	2.39
+S Order = 1	30.40	59.15	2.38	42.72	2.30
+CVs Fixed	37.88	52.74	2.19	53.31	2.29



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	350.71	4.20	10.45	657.39	4.27	30.40	59.15	1.87	59.40	n/a	4.88
Aneuploid A	710.65	6.28	54.90	1334.62	6.28	2.38	42.72	1.88	40.60	2.03	1.43

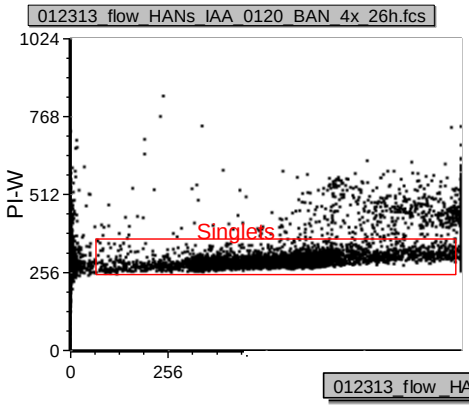
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=42.7, %G2=2.38
The S Phase confidence is poor
Note: intra-model error, inter-model error,

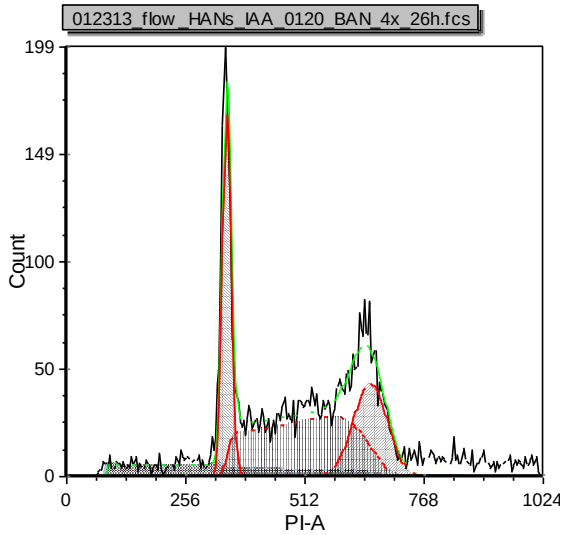
Experiment Statistics

Chi sq: 2.30
Average %S: 52.48
Average %S aneuploid: 42.72
BAD: 3.34
Number of cells: 5698.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

BAN 12 µM, initial cell loading 4×10^5 cells/well, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S2	30.31	44.57	2.97
SL CL S0	26.52	47.66	1.66
+G2/G1 Fixed	30.83	44.40	2.96
+Aggregates	27.29	46.80	1.66
+S Order = 1	25.20	49.50	2.93
+CVs Fixed	16.05	58.91	3.48



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	345.77	2.72	25.30	654.20	5.54	25.20	49.50	1.89	100.00	7.68

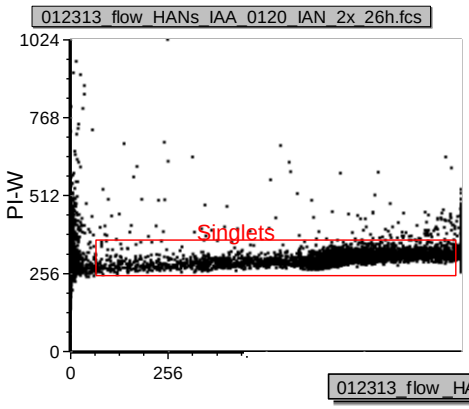
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=49.5, %G2=25.2
The S Phase confidence is poor
Note: intra-model error,

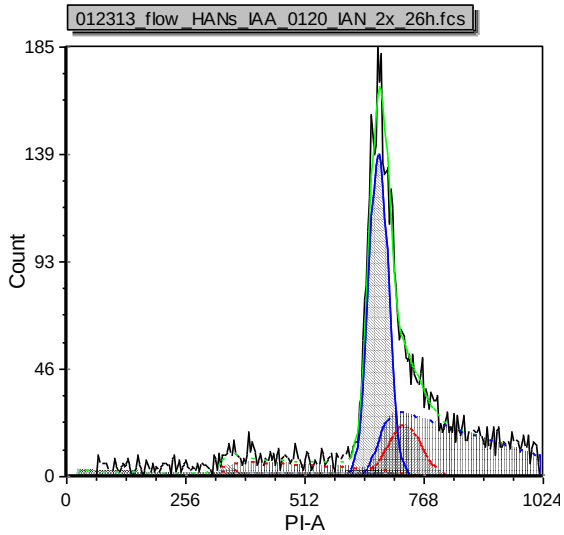
Experiment Statistics

Chi sq: 2.93
BAD: 7.68
Number of cells: 5203.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAN 6 µM, initial cell loading 2×10^5 cells/well, 26 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	0.00	0.00	1.23	0.00	10.17
SL CL S0	0.00	84.20	0.60	50.83	2.66
+G2/G1 Fixed	0.00	83.99	0.61	51.09	2.63
+Aggregates	0.00	84.21	0.60	51.57	2.64
+S Order = 1	49.91	44.90	0.65	50.37	1.67
+CVs Fixed	0.00	0.00	0.00	0.00	8.94



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	347.85	4.97	5.20	727.83	4.92	49.91	44.90	2.09	21.02	n/a	4.65
Aneuploid A	672.47	3.16	48.98	1243.14	3.16	0.65	50.37	1.85	78.98	1.93	1.34

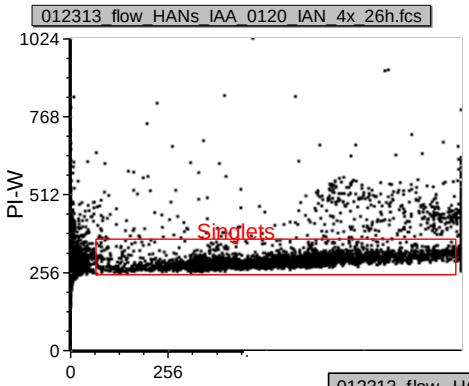
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=50.4, %G2=0.65
The S Phase confidence is poor
Note: intra-model error, inter-model error,

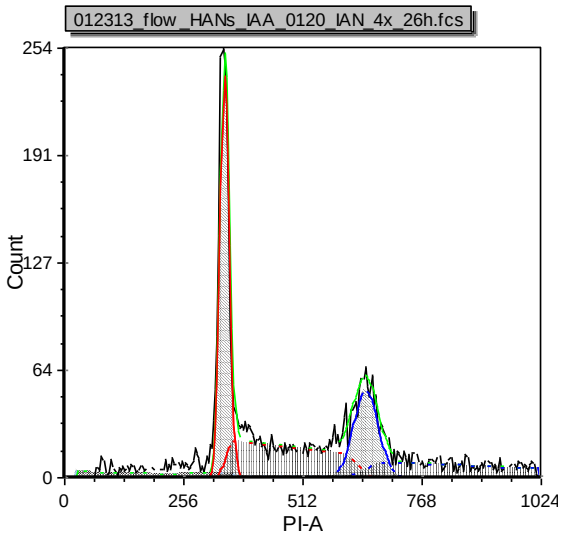
Experiment Statistics

Chi sq: 1.67
Average %S: 49.22
Average %S aneuploid: 50.37
BAD: 3.60
Number of cells: 5231.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

IAN 6 µM, initial cell loading 4×10^5 cells/well, 26 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	13.17	41.31	1.92	72.30	2.37
SL CL S0	0.20	47.48	1.62	58.80	2.48
+G2/G1 Fixed	6.49	44.07	1.90	63.55	2.42
+Aggregates	6.66	44.13	1.87	64.64	2.44
+S Order = 1	0.00	46.45	1.73	51.54	2.41
+CVs Fixed	13.52	41.19	1.93	73.38	2.39



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	345.37	2.89	53.55	633.58	2.89	0.00	46.45	1.83	60.36	n/a	3.52
Aneuploid A	649.02	4.03	46.73	1244.18	4.03	1.73	51.54	1.92	39.64	1.88	1.94

Interpretation

MultiCycle suggestions (a guideline only):
An aneuploid DNA content is observed.
The aneuploid %S=51.5, %G2=1.73
The S Phase confidence is poor
Note: intra-model error, inter-model error,

Experiment Statistics

Chi sq: 2.41
Average %S: 48.47
Average %S aneuploid: 51.54
BAD: 2.95
Number of cells: 5016.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

Appendix C.4 Cell cycle profiles of CHO cells at 0, 8, 17, 26, 34, 43, and 52 hour post-incubation. Treatment conditions are negative control, 900 µM CAN, 12 µM BAN, and 6 µM IAN. DNA contents in individual cells were measured with LSR II flow cytometer and the cell cycle distributions were calculated using the FCS Express software. A summary table, cell cycle distribution, and fitted curves as well as the fitting models are provided (raw data used for Figures 4.6 and 4.7).

Table C.4 Summary of cell cycle distribution (%) of the different treatment groups.

Negative control

Time (hours)	2N	2N to 4N	4N	4N to 8N	8N
0	36.21	42.61	21.17	0	0
8	31.97	37.51	30.53	0	0
17	31.18	39.93	28.89	0	0
26	22.23	48.56	29.22	0	0
34	28.28	47.99	23.74	0	0
43	33.60	43.51	22.89	0	0
52	31.40	45.97	22.63	0	0

CAN 900 µM

Time (hours)	2N	2N to 4N	4N	4N to 8N	8N
0	33.33	41.58	25.09	0	0
8	23.22	56.59	20.18	0	0
17	8.83	60.74	23.18	6.83	0.41
26	11.10	49.67	28.45	6.85	3.93
34	9.82	28.53	41.67	14.25	5.74
43	10.28	33.30	35.37	12.94	8.12
52	15.55	31.11	31.08	13.36	8.90

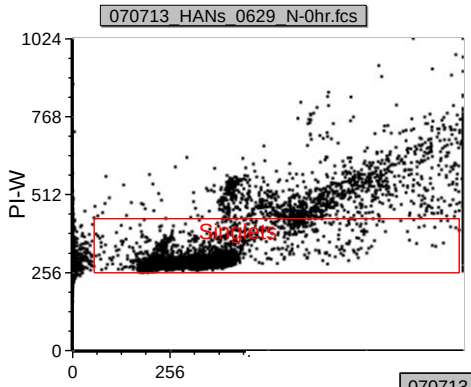
BAN 12 µM

Time (hours)	2N	2N to 4N	4N	4N to 8N	8N
0	29.32	49.32	21.36	0	0
8	17.41	54.20	28.39	0	0
17	7.41	27.75	53.06	7.60	4.20
26	10.58	35.49	31.54	17.08	5.32
34	11.04	27.28	31.36	16.03	14.29
43	10.22	31.70	23.73	21.63	12.71
52	23.83	23.20	22.97	20.90	9.10

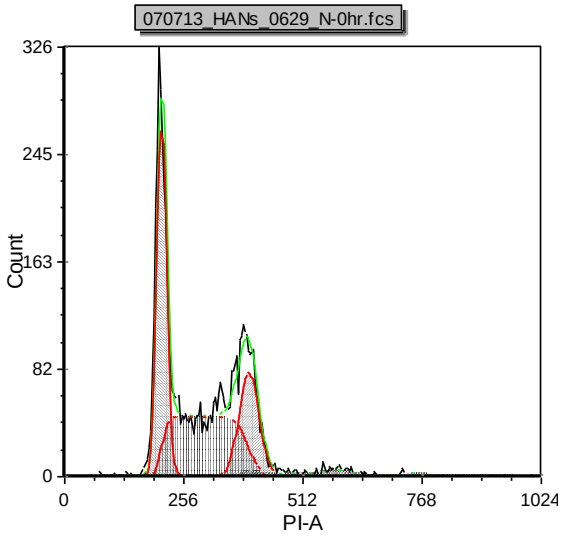
IAN 6 µM

Time (hours)	2N	2N to 4N	4N	4N to 8N	8N
0	27.43	50.68	21.90	0	0
8	11.55	33.67	54.77	0	0
17	2.55	4.47	75.76	7.56	9.67
26	9.09	18.36	53.67	10.84	8.05
34	5.92	18.08	21.63	30.83	23.55
43	6.63	26.21	18.02	36.59	12.56
52	13.06	30.39	25.02	23.42	8.11

Negative control, 0 hr, +aggregate model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV S0	22.52	41.63	1.83
SL CL S0	21.51	42.29	0.96
+G2/G1 Fixed	22.44	41.77	1.82
+Aggregates	21.17	42.61	0.96
+S Order = 1	22.47	41.74	1.84
+CVs Fixed	21.17	42.58	1.83



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	209.40	5.24	36.21	397.02	5.39	21.17	42.61	1.90	100.00	1.43

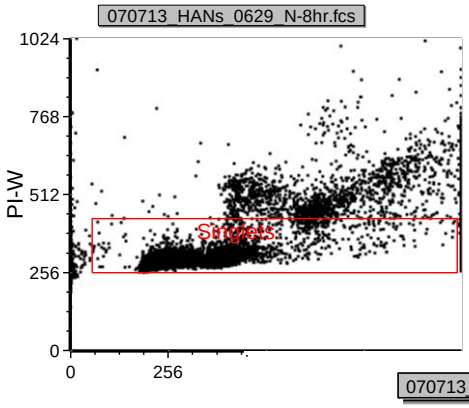
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=42.6, %G2=21.2
The S Phase confidence is good

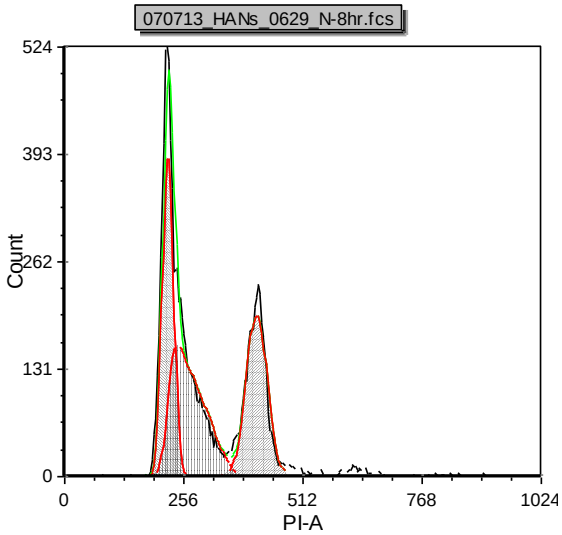
Experiment Statistics

Chi sq: 0.96
BAD: 1.43
Number of cells: 5572.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Negative control, 8 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV S0	24.68	27.10	5.95
SL CL S0	23.73	27.49	4.39
+G2/G1 Fixed	24.69	27.10	5.92
+Aggregates	23.42	27.86	4.39
+S Order = 1	30.53	37.51	3.08
+CVs Fixed	30.47	37.17	3.08



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	222.72	5.36	31.97	413.84	5.48	30.53	37.51	1.86	100.00	0.32

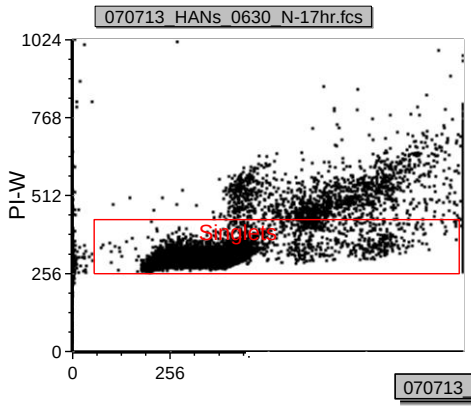
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=37.5, %G2=30.5
The S Phase confidence is good

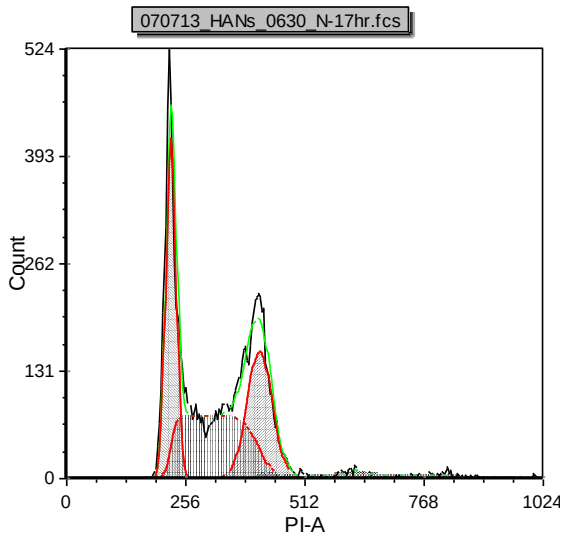
Experiment Statistics

Chi sq: 3.08
BAD: 0.32
Number of cells: 9914.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Negative control, 17 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV S0	29.87	39.24	3.38
SL CL S0	28.89	39.93	1.45
+G2/G1 Fixed	23.32	46.04	3.89
+Aggregates	22.11	46.96	2.03
+S Order = 1	35.52	35.44	3.30
+CVs Fixed	24.78	42.94	3.77



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	224.89	4.86	31.18	415.72	6.50	28.89	39.93	1.85	100.00	0.45

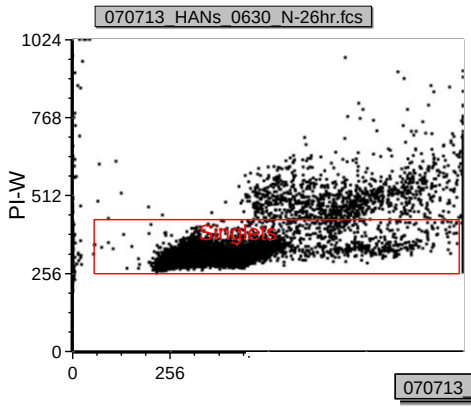
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=39.9, %G2=28.9
The S Phase confidence is good

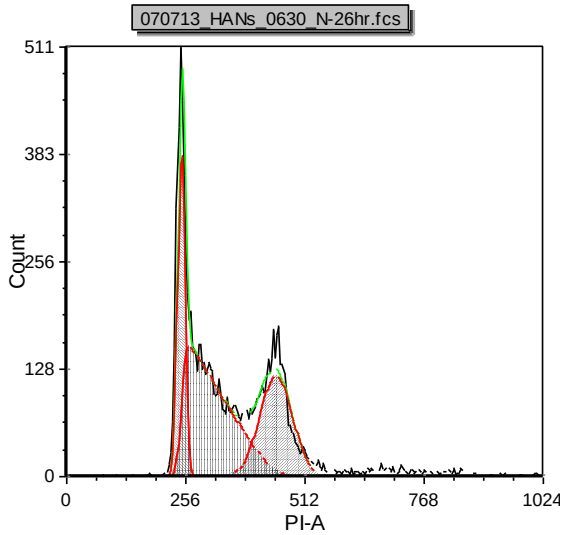
Experiment Statistics

Chi sq: 1.45
BAD: 0.45
Number of cells: 9998.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Negative control, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	28.66	48.88	3.71
SL CL S0	14.54	56.94	2.95
+G2/G1 Fixed	18.63	52.53	5.28
+Aggregates	16.96	53.98	3.11
+S Order = 1	29.22	48.56	3.63
+CVs Fixed	18.04	55.86	4.84



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	247.41	3.31	22.23	450.96	7.71	29.22	48.56	1.82	100.00	0.00

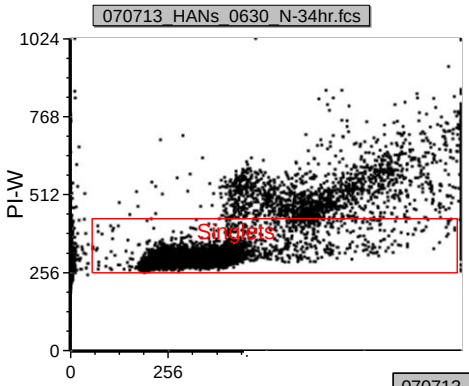
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=48.6, %G2=29.2
The S Phase confidence is poor
Note: intra-model error,

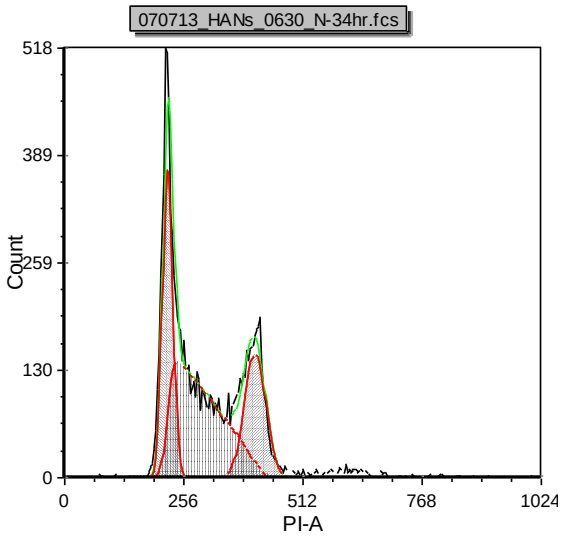
Experiment Statistics

Chi sq: 3.63
BAD: 0.00
Number of cells: 9896.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Negative control, 34 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	25.60	45.16	3.33
SL CL S0	14.69	51.19	2.05
+G2/G1 Fixed	19.96	45.29	4.08
+Aggregates	18.74	46.16	2.59
+S Order = 1	23.74	47.99	2.98
+CVs Fixed	22.11	48.68	2.99



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	222.15	4.94	28.28	410.47	5.59	23.74	47.99	1.85	100.00	0.69

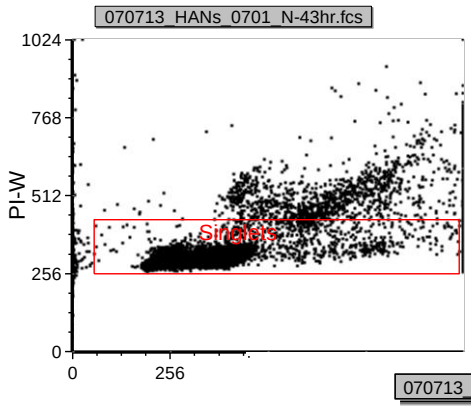
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=48., %G2=23.7
The S Phase confidence is poor
Note: intra-model error,

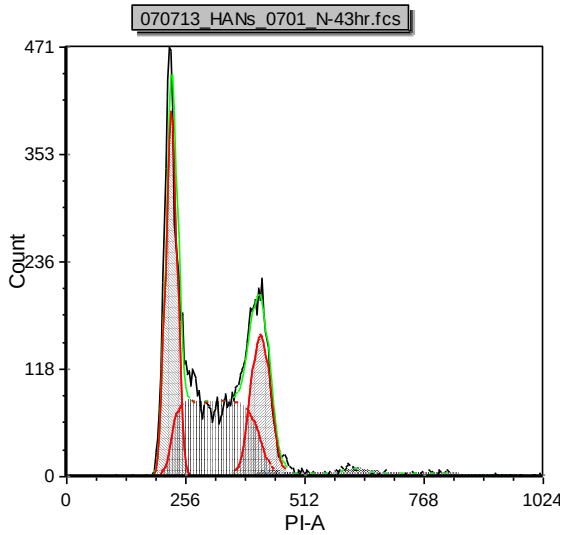
Experiment Statistics

Chi sq: 2.98
BAD: 0.69
Number of cells: 9808.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Negative control, 43 hr, +aggregates model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV S0	24.14	42.56	3.62
SL CL S0	23.18	43.19	1.72
+G2/G1 Fixed	24.12	42.59	3.60
+Aggregates	22.89	43.51	1.71
+S Order = 1	31.33	39.09	3.35
+CVs Fixed	27.02	41.39	3.47



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	225.78	5.38	33.60	417.91	5.12	22.89	43.51	1.85	100.00	0.38

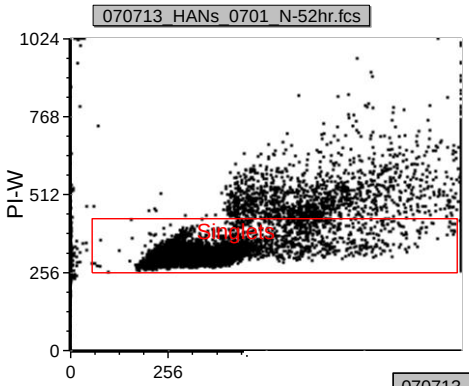
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=43.5, %G2=22.9
The S Phase confidence is good

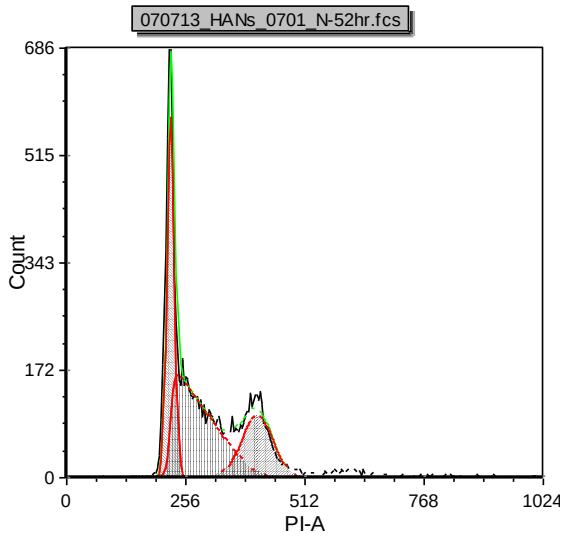
Experiment Statistics

Chi sq: 1.71
BAD: 0.38
Number of cells: 9978.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Negative control, 52 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	15.21	50.09	5.27
SL CL S0	8.27	53.58	2.51
+G2/G1 Fixed	14.67	47.03	5.31
+Aggregates	11.92	48.96	3.05
+S Order = 1	22.63	45.97	3.53
+CVs Fixed	11.73	53.32	4.54



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	224.15	3.58	31.40	410.36	8.21	22.63	45.97	1.83	100.00	0.59

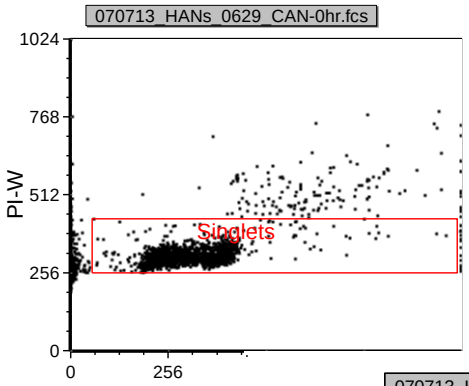
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=46., %G2=22.6
The S Phase confidence is poor
Note: intra-model error,

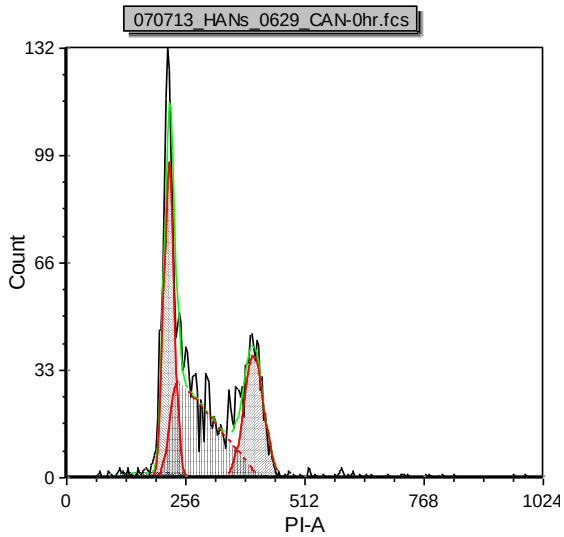
Experiment Statistics

Chi sq: 3.53
BAD: 0.59
Number of cells: 10043.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

CAN 900 µM, 0 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV S0	17.28	43.41	1.00
SL CL S0	16.86	43.62	0.95
+G2/G1 Fixed	17.23	43.46	0.99
+Aggregates	16.71	43.80	0.95
+S Order = 1	25.09	41.58	0.87
+CVs Fixed	24.28	41.85	0.87



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	220.83	4.97	33.33	403.00	5.30	25.09	41.58	1.83	100.00	1.90

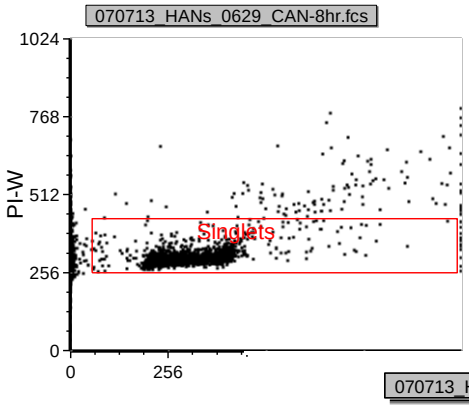
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=41.6, %G2=25.1
The S Phase confidence is good

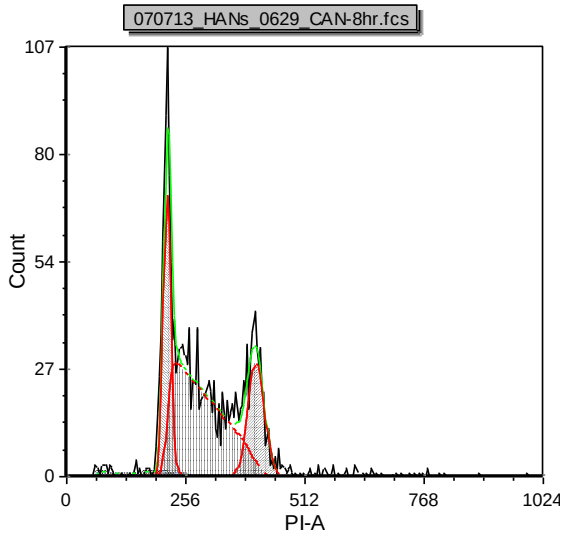
Experiment Statistics

Chi sq: 0.87
BAD: 1.90
Number of cells: 2241.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

CAN 900 µM, 8 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV S0	12.94	58.56	0.97
SL CL S0	12.39	58.91	0.87
+G2/G1 Fixed	12.92	58.59	0.97
+Aggregates	12.25	59.05	0.86
+S Order = 1	20.18	56.59	0.80
+CVs Fixed	18.98	57.24	0.80



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	217.00	3.80	23.22	408.37	4.36	20.18	56.59	1.88	100.00	1.94

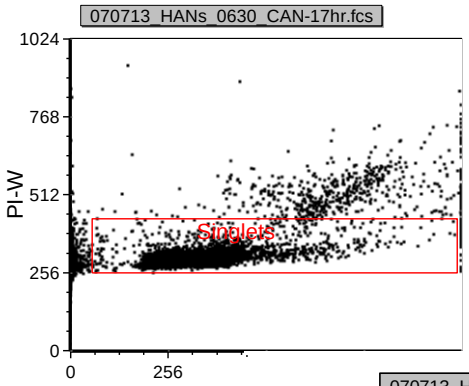
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=56.6, %G2=20.2
The S Phase confidence is good

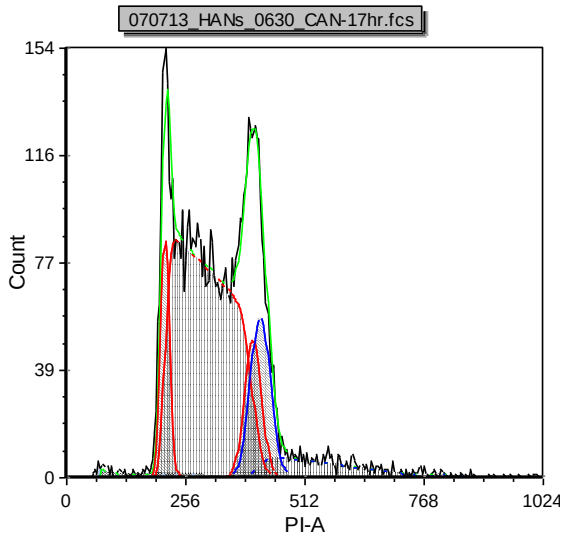
Experiment Statistics

Chi sq: 0.80
BAD: 1.94
Number of cells: 1788.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

CAN 900 µM, 17 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S0	0.00	84.29	0.00	18.74	1.14
SL CL S0	0.00	84.28	0.00	18.65	1.15
+G2/G1 Fixed	0.00	84.28	0.00	18.65	1.13
+Aggregates	0.00	84.28	0.00	18.65	1.14
+S Order = 1	12.26	76.60	1.99	32.99	0.80
+CVs Fixed	8.99	78.48	1.71	27.44	0.82



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	212.27	4.55	11.14	401.48	4.57	12.26	76.60	1.89	79.30	n/a	1.04
Aneuploid A	417.43	5.17	65.02	805.01	5.17	1.99	32.99	1.93	20.70	1.97	0.54

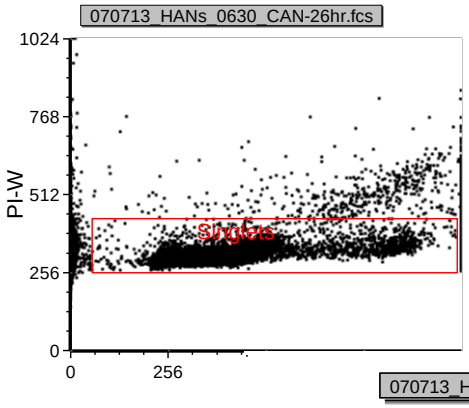
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=33., %G2=1.99
The S Phase confidence is fair
Note: % cells, inter-model error,

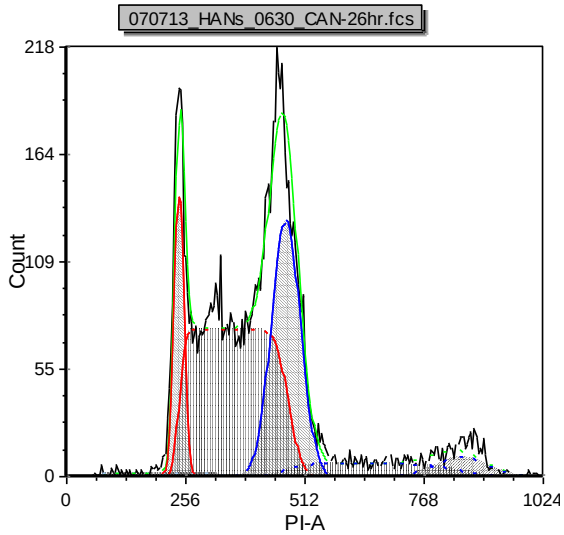
Experiment Statistics

Chi sq: 0.80
Average %S: 67.58
Average %S aneuploid: 32.99
BAD: 0.90
Number of cells: 6047.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

CAN 900 µM, 26 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL S0	0.00	81.70	9.84	17.59	1.32
SL CL S0	0.00	81.73	10.13	18.20	1.35
+G2/G1 Fixed	0.00	81.44	0.00	26.54	1.70
+Aggregates	5.98	73.70	9.24	16.06	1.36
+S Order = 1	0.00	81.73	10.02	17.45	1.33
+CVs Fixed	0.00	0.00	1.04	98.96	20.91



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	242.28	4.55	18.27	479.60	4.55	0.00	81.73	1.98	60.77	n/a	1.04
Aneuploid A	472.50	6.58	72.53	853.45	6.58	10.02	17.45	1.81	39.23	1.95	0.66

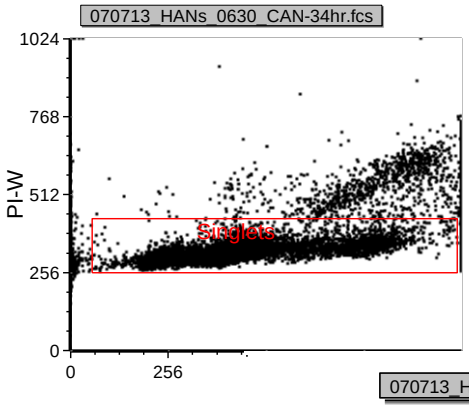
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=17.4, %G2=10.
The S Phase confidence is fair
Note: inter-model error,

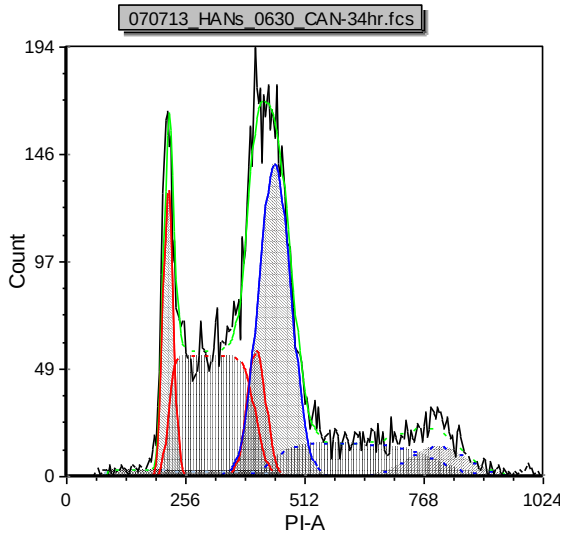
Experiment Statistics

Chi sq: 1.33
Average %S: 56.52
Average %S aneuploid: 17.45

CAN 900 µM, 34 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV S0	16.69	61.83	10.69	26.27	1.36
SL CL S0	25.57	55.45	10.52	29.48	1.32
+G2/G1 Fixed	14.96	63.36	4.06	33.19	1.64
+Aggregates	7.69	65.73	10.24	21.38	1.50
+S Order = 1	17.17	61.63	10.68	26.53	1.37
+CVs Fixed	78.60	0.00	0.00	24.96	7.94



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	219.15	4.95	21.21	408.87	4.94	17.17	61.63	1.87	46.30	n/a	2.67
Aneuploid A	448.42	7.69	62.79	802.91	7.69	10.68	26.53	1.79	53.70	2.05	1.21

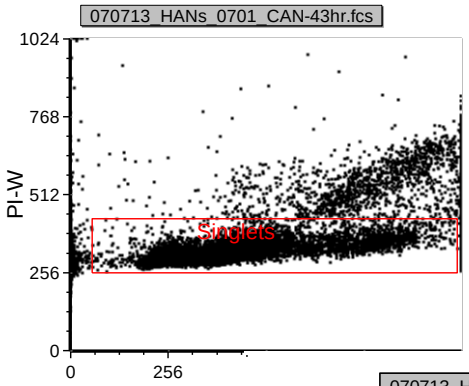
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=26.5, %G2=10.7
The S Phase confidence is fair
Note: inter-model error,

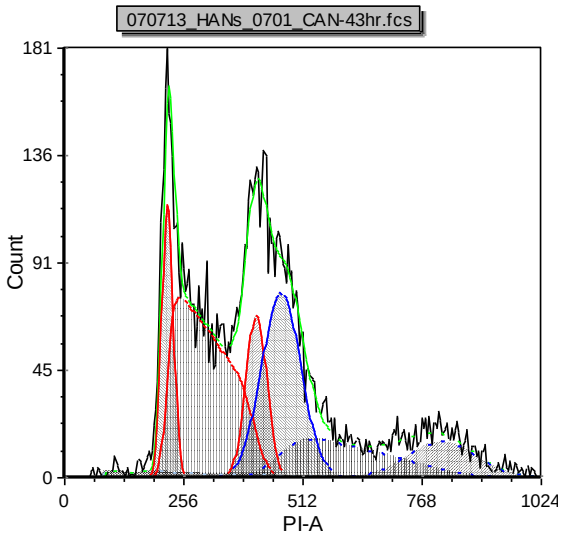
Experiment Statistics

Chi sq: 1.37
Average %S: 42.78
Average %S aneuploid: 26.53
BAD: 1.84
Number of cells: 9620.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

CAN 900 µM, 43 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	19.25	62.39	12.69	32.71	1.42
SL CL S0	20.63	58.58	13.87	32.14	1.47
+G2/G1 Fixed	16.46	60.11	8.14	35.45	1.68
+Aggregates	16.09	60.44	8.60	35.65	1.68
+S Order = 1	20.57	60.70	17.98	28.67	1.25
+CVs Fixed	30.16	52.02	18.19	39.09	2.42



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	221.74	5.49	18.73	413.10	5.48	20.57	60.70	1.86	54.86	n/a	2.11
Aneuploid A	466.75	9.07	53.35	814.81	9.07	17.98	28.67	1.75	45.14	2.11	1.26

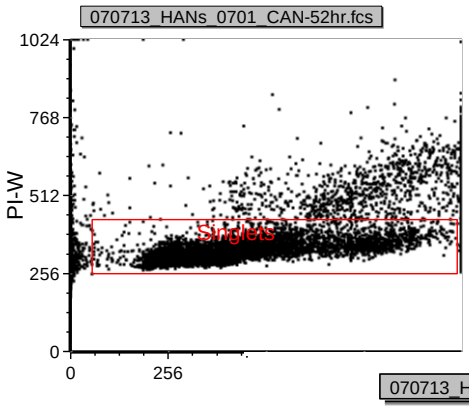
Interpretation

MultiCycle suggestions (a guideline only):
An aneuploid DNA content is observed.
The aneuploid %S=28.7, %G2=18.
The S Phase confidence is good

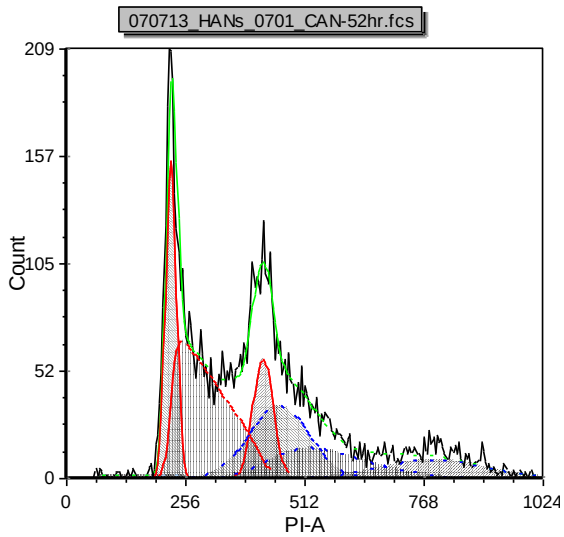
Experiment Statistics

Chi sq: 1.25
Average %S: 46.24
Average %S aneuploid: 28.67
BAD: 1.67
Number of cells: 9087.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

CAN 900 µM, 52 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	15.27	56.68	21.54	53.13	1.12
SL CL S0	17.36	55.61	13.17	40.16	1.36
+G2/G1 Fixed	18.22	53.89	0.00	52.32	1.46
+Aggregates	19.04	53.46	0.00	55.26	1.50
+S Order = 1	19.12	53.93	21.04	31.56	1.26
+CVs Fixed	27.18	48.96	0.00	75.29	2.57



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	224.91	5.14	26.96	423.43	5.13	19.12	53.93	1.88	57.68	n/a	1.38
Aneuploid A	458.21	14.14	47.39	792.64	14.14	21.04	31.56	1.73	42.32	2.04	1.01

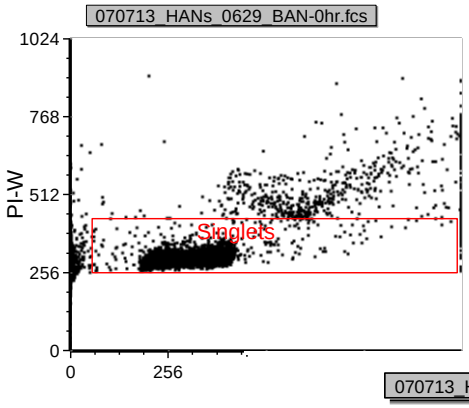
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=31.6, %G2=21.
The S Phase confidence is fair
Note: inter-model error,

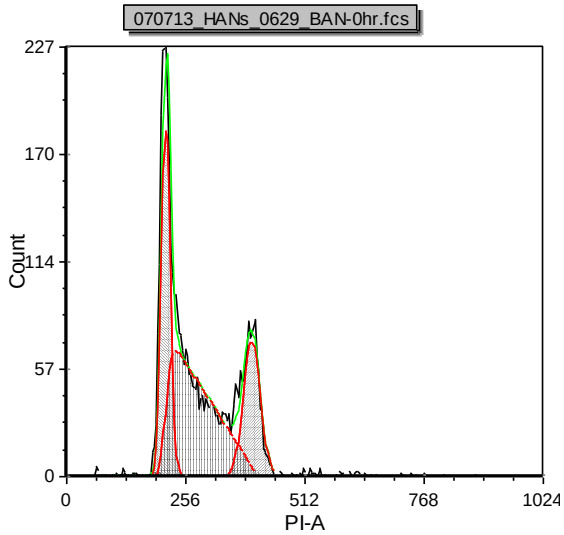
Experiment Statistics

Chi sq: 1.26
Average %S: 44.46
Average %S aneuploid: 31.56
BAD: 1.20
Number of cells: 7559.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

BAN 12 µM, 0 hr, +CVs fixed model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV S0	13.92	49.44	1.28
SL CL S0	13.39	49.75	1.05
+G2/G1 Fixed	13.94	49.43	1.28
+Aggregates	13.24	49.92	1.05
+S Order = 1	21.75	49.26	0.81
+CVs Fixed	21.36	49.32	0.80



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	214.04	4.56	29.32	398.97	4.56	21.36	49.32	1.86	100.00	0.82

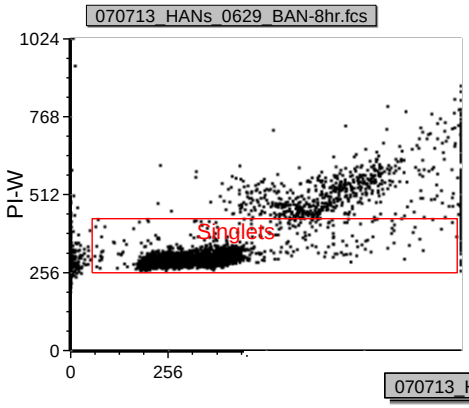
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=49.3, %G2=21.4
The S Phase confidence is good

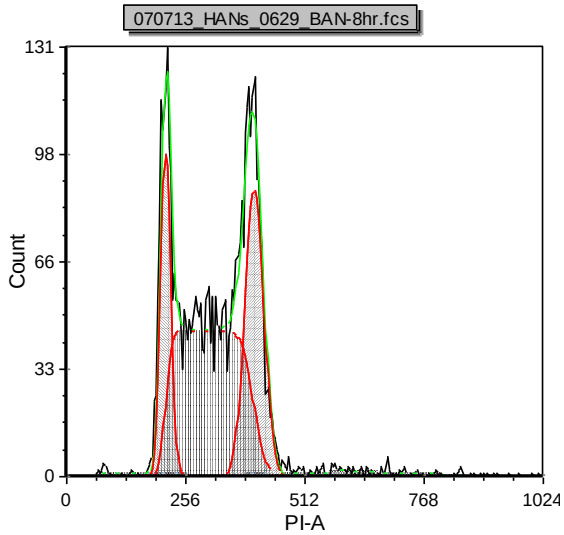
Experiment Statistics

Chi sq: 0.80
BAD: 0.82
Number of cells: 4082.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAN 12 µM, 8 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV S0	28.69	53.96	1.10
SL CL S0	28.39	54.20	0.78
+G2/G1 Fixed	31.03	51.25	1.16
+Aggregates	30.73	51.48	0.84
+S Order = 1	29.43	53.57	1.10
+CVs Fixed	29.44	53.56	1.10



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	213.69	5.16	17.41	404.49	5.02	28.39	54.20	1.89	100.00	1.15

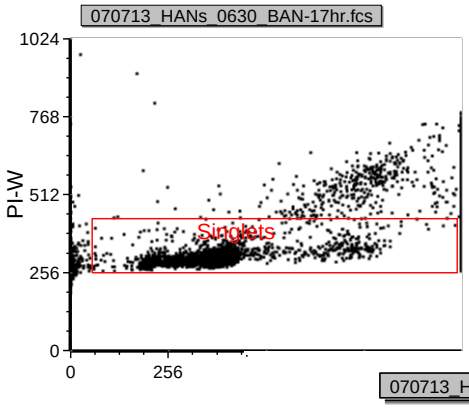
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=54.2, %G2=28.4
The S Phase confidence is good

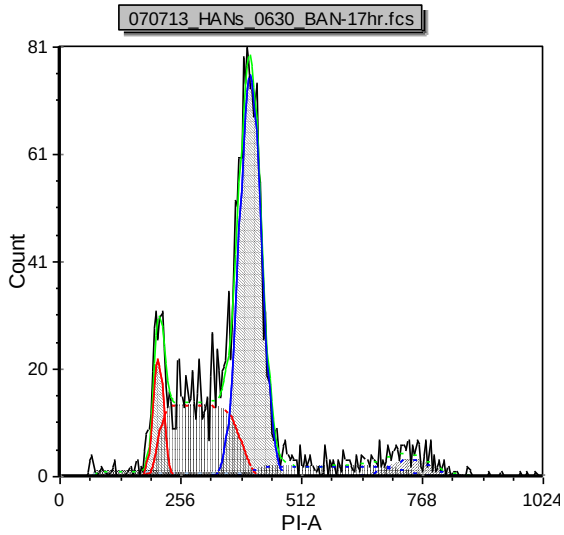
Experiment Statistics

Chi sq: 0.78
BAD: 1.15
Number of cells: 4215.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAN 12 µM, 17 hr, +aggregates model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL S0	3.61	78.44	7.72	13.58	0.80
SL CL S0	12.51	70.88	7.78	12.07	0.83
+G2/G1 Fixed	28.97	57.78	4.39	24.00	0.87
+Aggregates	0.00	78.92	6.47	11.72	0.79
+S Order = 1	3.65	78.42	7.71	13.58	0.81
+CVs Fixed	0.00	78.84	8.08	12.74	0.81



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	208.67	5.42	21.08	385.28	5.40	0.00	78.92	1.85	35.16	n/a	3.20
Aneuploid A	404.47	5.82	81.82	746.65	5.82	6.47	11.72	1.85	64.85	1.94	2.22

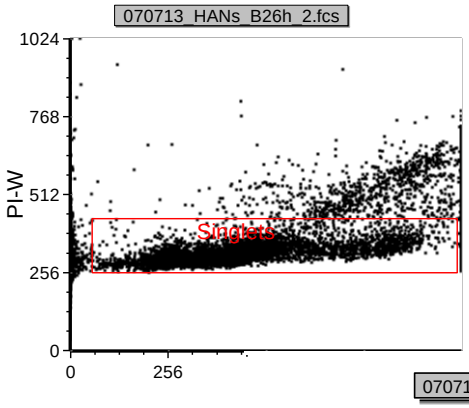
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=11.7, %G2=6.47
The S Phase confidence is fair
Note: inter-model error,

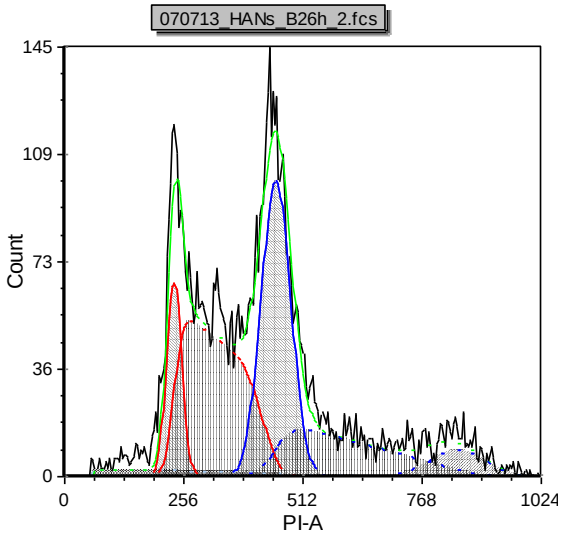
Experiment Statistics

Chi sq: 0.79
Average %S: 35.35
Average %S aneuploid: 11.72
BAD: 2.48
Number of cells: 2330.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

BAN 12 µM, 26 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	0.00	78.96	12.71	31.09	1.44
SL CL S0	8.20	70.16	5.93	41.73	1.75
+G2/G1 Fixed	0.00	73.86	7.59	28.87	1.99
+Aggregates	4.65	70.84	7.13	31.96	1.75
+S Order = 1	0.00	77.03	9.87	31.66	1.75
+CVs Fixed	0.00	76.69	10.10	31.67	1.72



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	236.04	6.88	22.97	429.41	6.88	0.00	77.03	1.82	46.07	n/a	2.49
Aneuploid A	455.43	6.94	58.47	848.62	6.94	9.87	31.66	1.86	53.94	1.93	1.29

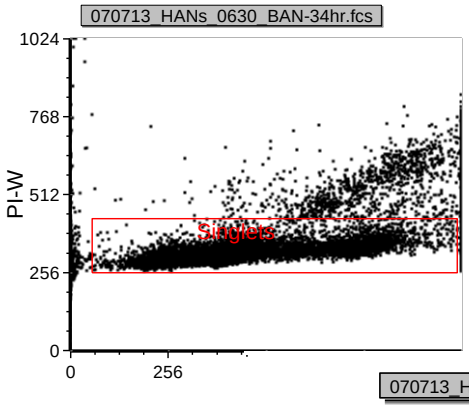
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=31.7, %G2=9.87
The S Phase confidence is fair
Note: inter-model error,

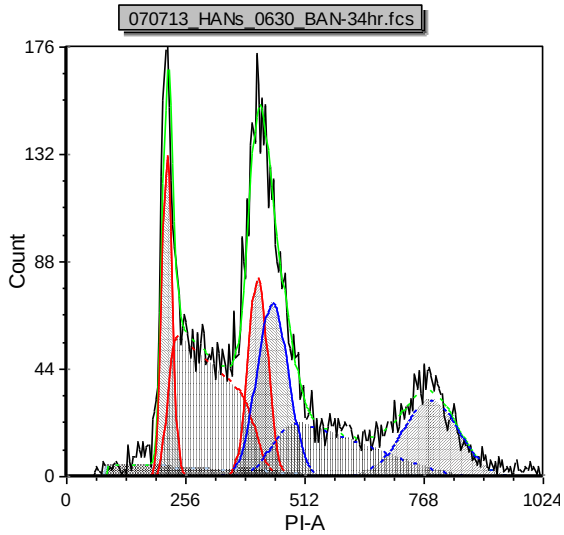
Experiment Statistics

Chi sq: 1.75
Average %S: 52.56
Average %S aneuploid: 31.66
BAD: 1.85
Number of cells: 7235.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

BAN 12 µM, 34 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV S0	29.59	49.25	26.53	39.16	1.60
SL CL S0	41.64	39.33	100.00	0.00	10.70
+G2/G1 Fixed	28.24	50.24	2.75	63.48	2.34
+Aggregates	0.00	62.44	17.67	13.04	3.34
+S Order = 1	25.42	53.08	29.40	32.98	1.48
+CVs Fixed	36.90	44.05	28.96	44.10	1.60



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	216.79	5.08	21.49	412.20	5.09	25.42	53.08	1.90	51.39	n/a	4.34
Aneuploid A	443.85	7.57	37.63	784.85	7.57	29.40	32.98	1.77	48.61	2.05	2.76

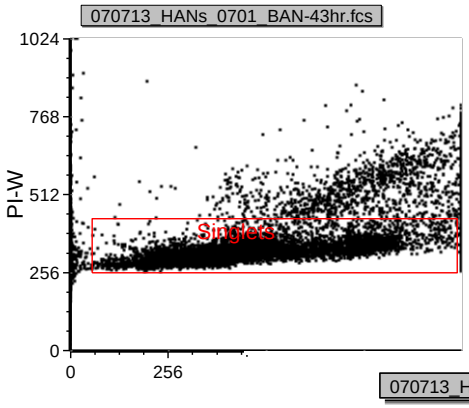
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=33., %G2=29.4
The S Phase confidence is fair
Note: inter-model error,

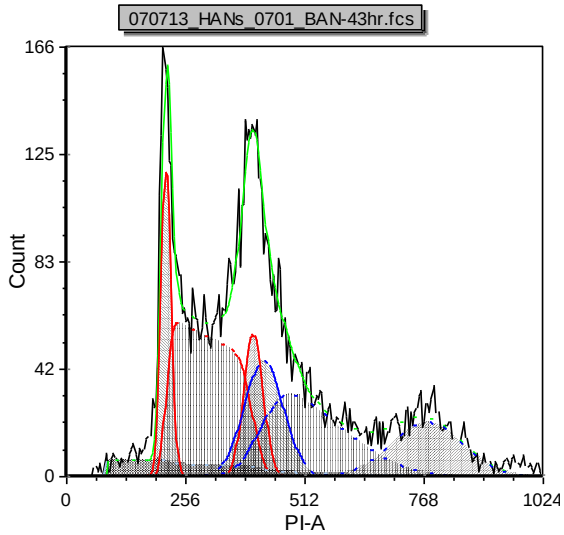
Experiment Statistics

Chi sq: 1.48
Average %S: 43.31
Average %S aneuploid: 32.98
BAD: 3.43
Number of cells: 8918.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

BAN 12 µM, 43 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV S0	22.84	57.85	19.25	39.20	1.63
SL CL S0	27.69	54.27	15.05	46.31	1.55
+G2/G1 Fixed	18.06	61.52	5.24	55.02	1.85
+Aggregates	12.50	58.24	15.32	24.34	2.33
+S Order = 1	17.62	62.30	25.88	44.05	1.50
+CVs Fixed	30.41	50.94	23.40	55.18	1.63



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	214.49	5.12	20.08	402.41	5.17	17.62	62.30	1.88	50.89	n/a	5.44
Aneuploid A	424.42	9.91	30.07	779.86	9.91	25.88	44.05	1.84	49.11	1.98	3.66

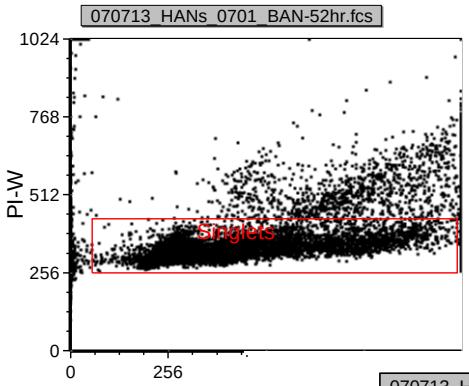
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=44.1, %G2=25.9
The S Phase confidence is fair
Note: inter-model error,

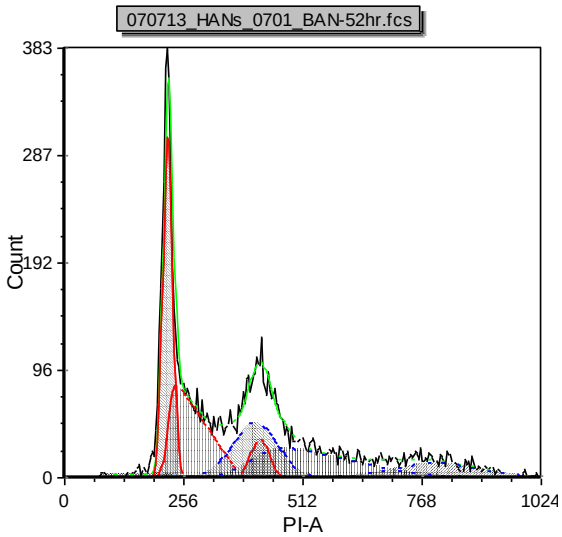
Experiment Statistics

Chi sq: 1.50
Average %S: 53.34
Average %S aneuploid: 44.05
BAD: 4.54
Number of cells: 8831.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

BAN 12 µM, 52 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	10.35	39.48	22.09	24.55	1.53
SL CL S0	6.78	57.64	0.00	100.00	3.91
+G2/G1 Fixed	11.24	44.69	9.52	52.79	2.07
+Aggregates	11.77	47.87	0.00	100.00	4.82
+S Order = 1	9.79	44.50	19.02	43.67	1.33
+CVs Fixed	16.54	45.31	0.48	75.51	1.64



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	222.41	4.62	45.71	420.63	4.62	9.79	44.50	1.89	52.14	n/a	2.10
Aneuploid A	410.39	11.73	37.32	804.24	11.73	19.02	43.67	1.96	47.86	1.85	1.69

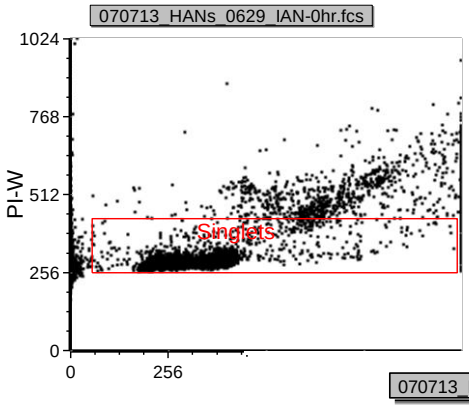
Interpretation

MultiCycle suggestions (a guideline only):
An aneuploid DNA content is observed.
The average %S=44.1, %G2=14.2
The S Phase confidence is fair
Note: inter-model error,

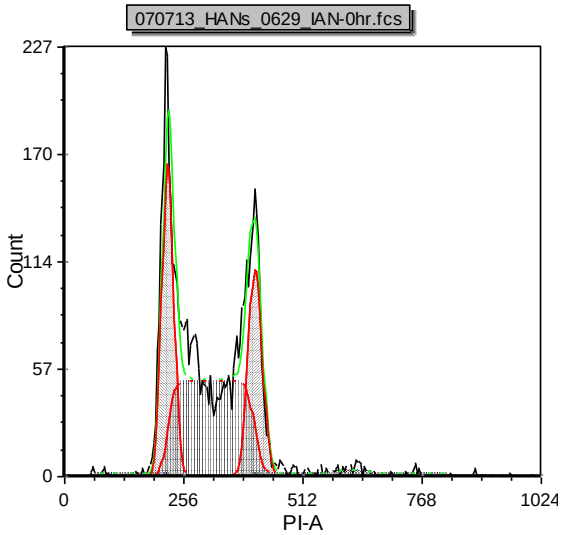
Experiment Statistics

Chi sq: 1.33
Average %S: 44.10
Average %S aneuploid: 43.67
BAD: 1.95
Number of cells: 8775.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

IAN 6 µM, 0 hr, +aggregates model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV S0	22.46	50.28	2.07
SL CL S0	21.92	50.67	1.47
+G2/G1 Fixed	22.47	50.26	2.06
+Aggregates	21.90	50.68	1.46
+S Order = 1	28.83	49.31	1.83
+CVs Fixed	30.01	48.89	1.83



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	222.40	5.62	27.43	410.77	3.70	21.90	50.68	1.85	100.00	1.20

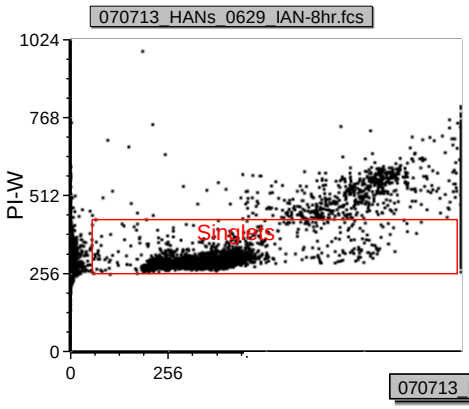
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=50.7, %G2=21.9
The S Phase confidence is good

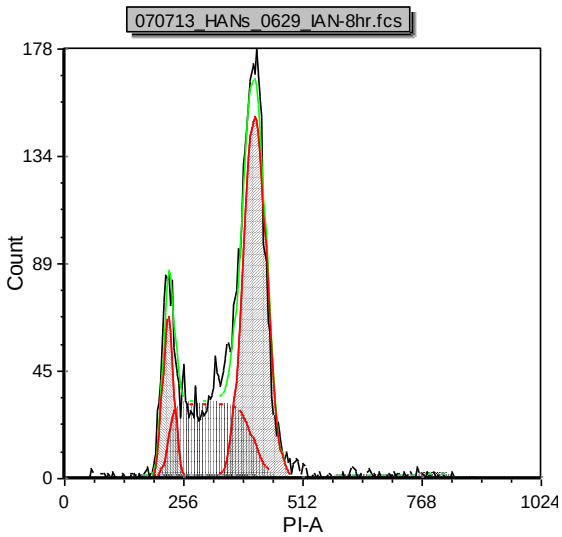
Experiment Statistics

Chi sq: 1.46
BAD: 1.20
Number of cells: 5319.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAN 6 µM, 8 hr, +aggregates model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV S0	55.73	32.64	1.44
SL CL S0	55.45	32.90	1.05
+G2/G1 Fixed	54.98	33.50	1.44
+Aggregates	54.77	33.67	1.05
+S Order = 1	51.26	36.11	1.40
+CVs Fixed	51.81	35.19	1.40



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	223.23	5.27	11.55	409.84	6.13	54.77	33.67	1.84	100.00	1.79

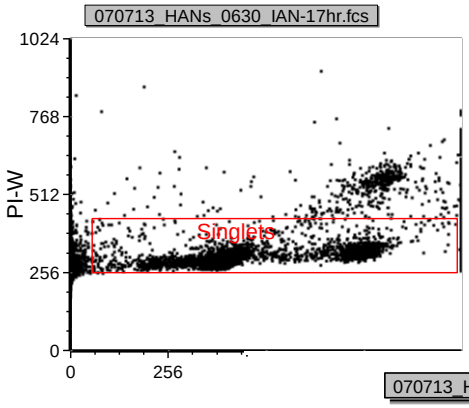
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=33.7, %G2=54.8
The S Phase confidence is good

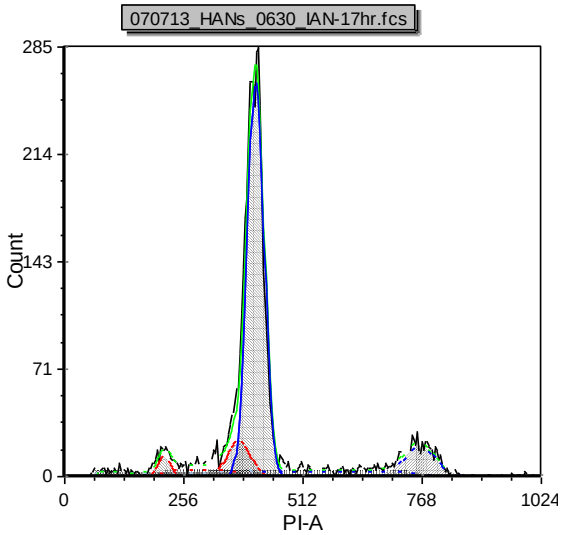
Experiment Statistics

Chi sq: 1.05
BAD: 1.79
Number of cells: 4713.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAN 6 µM, 17 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL S0	53.95	29.29	11.41	8.92	1.17
SL CL S0	27.60	56.30	11.42	8.47	1.41
+G2/G1 Fixed	0.00	78.24	3.67	12.38	2.36
+Aggregates	26.46	53.15	10.80	8.15	1.42
+S Order = 1	53.98	29.30	11.41	8.92	1.18
+CVs Fixed	0.00	0.00	0.93	89.13	214.18



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	214.17	6.56	16.72	374.86	6.56	53.98	29.30	1.75	15.27	n/a	16.62
Aneuploid A	411.78	4.37	79.67	766.56	4.37	11.41	8.92	1.86	84.74	1.92	1.98

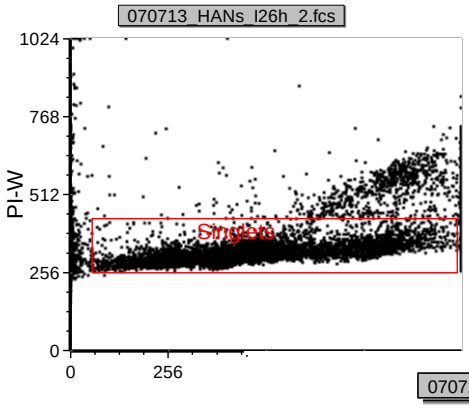
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=8.91, %G2=11.4
The S Phase confidence is fair
Note: inter-model error,

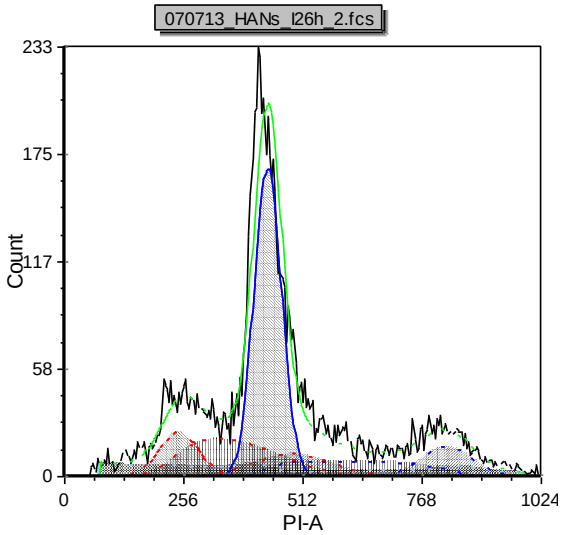
Experiment Statistics

Chi sq: 1.18
Average %S: 12.03
Average %S aneuploid: 8.92
BAD: 4.29
Number of cells: 4881.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

IAN 6 µM, 26 hr, sliced background model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV S1	92.57	0.00	35.06	42.30	3.29
SL CL S0	25.07	50.13	12.70	17.11	1.91
+G2/G1 Fixed	65.06	29.15	100.00	0.00	4.91
+Aggregates	33.62	64.98	100.00	0.00	15.92
+S Order = 1	92.46	0.68	41.04	34.87	3.10
+CVs Fixed	90.31	0.50	11.12	77.82	7.03



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	246.12	17.15	24.81	492.23	17.15	25.07	50.13	2.00	36.63	n/a	6.86
Aneuploid A	437.99	6.76	70.19	825.15	6.76	12.70	17.11	1.88	63.38	1.78	8.82

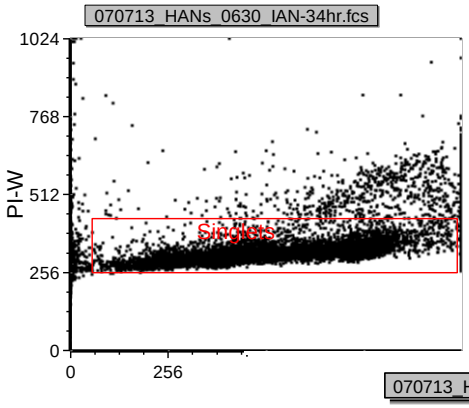
Interpretation

MultiCycle suggestions (a guideline only):
An aneuploid DNA content is observed.
The average %S=29.2, %G2=17.2
The S Phase confidence is fair
Note: inter-model error,

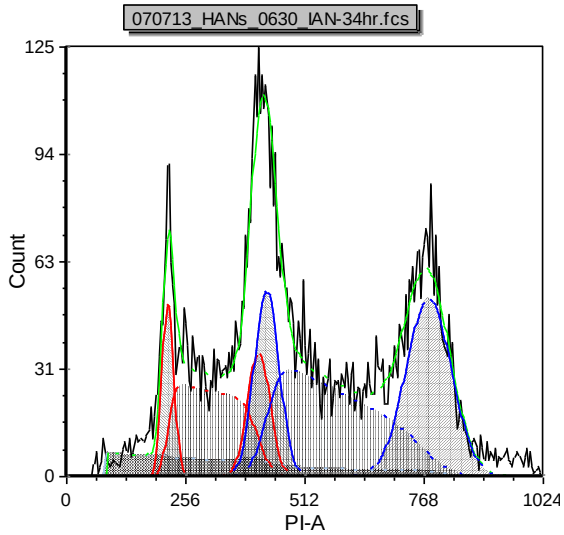
Experiment Statistics

Chi sq: 1.91
Average %S: 29.20
Average %S aneuploid: 17.11
BAD: 8.87
Number of cells: 8446.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

IAN 6 µM, 34 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL S0	27.03	54.27	30.45	45.87	2.47
SL CL S0	0.00	99.32	0.00	88.98	1164720.00
+G2/G1 Fixed	27.29	54.64	6.97	73.72	3.61
+Aggregates	13.08	61.36	28.21	38.02	2.12
+S Order = 1	25.36	56.24	34.71	45.44	1.91
+CVs Fixed	0.00	0.00	45.70	0.00	8.52



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	218.21	5.79	18.40	413.79	5.83	25.36	56.24	1.90	32.15	n/a	11.42
Aneuploid A	431.18	6.22	19.86	780.34	6.22	34.71	45.44	1.81	67.85	1.98	5.73

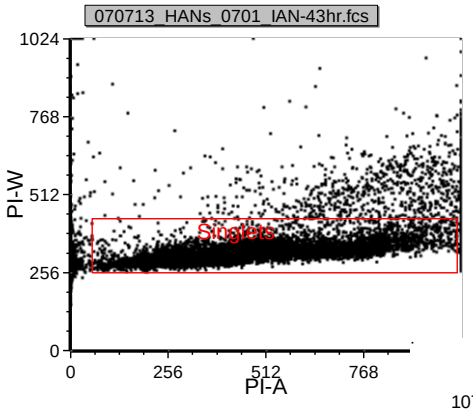
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=45.4, %G2=34.7
The S Phase confidence is fair
Note: inter-model error,

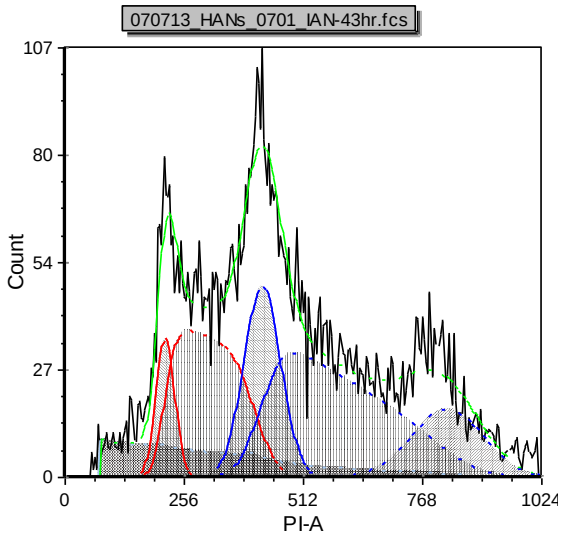
Experiment Statistics

Chi sq: 1.91
Average %S: 48.91
Average %S aneuploid: 45.44
BAD: 7.61
Number of cells: 7861.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

IAN 6 µM, 43 hr, +CVs fixed model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	9.08	67.79	23.97	38.49	1.90
SL CL S0	16.80	67.93	18.94	55.02	1.64
+G2/G1 Fixed	3.98	67.87	25.00	33.58	1.85
+Aggregates	4.69	73.59	14.50	43.40	1.84
+S Order = 1	0.00	83.57	17.87	65.43	1.69
+CVs Fixed	0.00	79.82	18.70	54.48	1.59



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	215.78	9.34	20.18	412.64	9.34	0.00	79.82	1.91	32.84	n/a	11.94
Aneuploid A	424.90	9.34	26.83	824.57	9.34	18.70	54.48	1.94	67.16	1.97	6.60

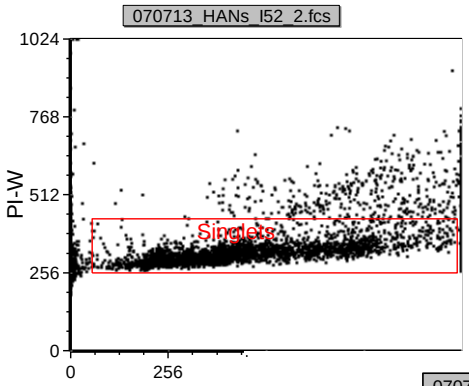
Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=54.5, %G2=18.7
The S Phase confidence is fair
Note: inter-model error,

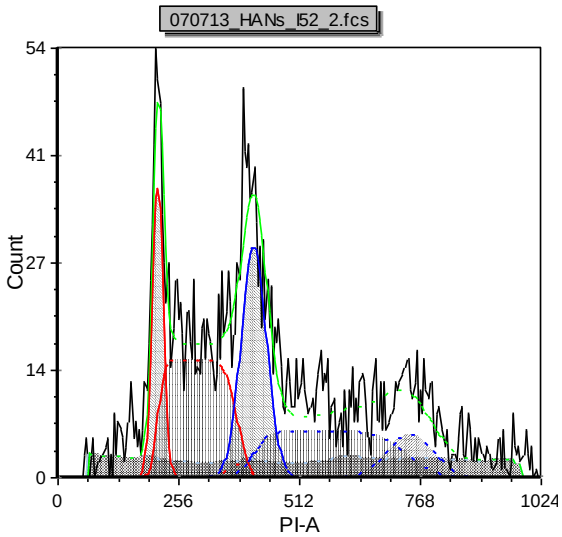
Experiment Statistics

Chi sq: 1.59
Average %S: 62.80
Average %S aneuploid: 54.48
BAD: 8.76
Number of cells: 7860.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

IAN 6 µM, 52 hr, +aggregates model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	0.00	68.86	20.66	38.94	1.75
SL CL S0	3.30	75.58	15.69	60.34	1.39
+G2/G1 Fixed	0.00	69.06	19.51	40.40	1.75
+Aggregates	2.43	68.24	14.62	42.22	1.54
+S Order = 1	0.00	69.22	14.41	48.05	1.65
+CVs Fixed	0.00	0.00	23.95	50.95	3.52



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	212.07	5.55	29.33	381.71	5.55	2.43	68.24	1.80	44.53	n/a	9.56
Aneuploid A	416.26	6.53	43.16	749.24	6.53	14.62	42.22	1.80	55.47	1.96	17.86

Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=42.2, %G2=14.6
The S Phase confidence is poor
Note: intra-model error, inter-model error,

Experiment Statistics

Chi sq: 1.54
Average %S: 53.81
Average %S aneuploid: 42.22
BAD: 13.04
Number of cells: 2881.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

Appendix C.5 CHO cell viability and cell numbers during post-incubation after treated without or with 900 µM CAN, 12 µM BAN, 6 µM IAN, 50 µM AraC, or 10 mM EMS (raw data used for Figure 4.8).

Cell number (# per well)

Time (hours)	Negative control	CAN 900 µM	BAN 12 µM	IAN 6 µM	AraC 50 µM	EMS 10 mM
0	358470	320600	327320	339080	340340	543410
8	481530	277760	298900	335580	–	–
17	722475	242700	214050	215625	–	–
26	1277550	171225	235350	268725	489750	708400
34	1771800	195075	240675	212850	–	–
43	2883000	247275	338100	322275	–	–
52	4164000	227400	427350	372525	541875	1032500

Viability (%)

Time (hours)	Negative control	CAN 900 µM	BAN 12 µM	IAN 6 µM	AraC 50 µM	EMS 10 mM
0	97.60	96.36	96.38	94.81	91.62	99.52
8	89.07	92.96	94.70	98.51	–	–
17	94.38	93.78	92.18	95.22	–	–
26	94.48	94.59	91.49	96.89	90.24	96.77
34	91.67	95.81	97.60	94.37	–	–
43	96.69	93.03	88.52	94.62	–	–
52	97.30	95.80	94.09	92.47	95.10	97.37

Appendix C.6 Cell cycle profiles of CHO cells treated without or with 10 mM EMS for 4 hours and harvested at 0, 26, and 52 hour post-incubation. DNA contents in individual cells were measured with LSR II flow cytometer and the cell cycle distributions were calculated using the FCS Express software. A summary table, cell cycle distribution, and fitted curves as well as the fitting models are provided (raw data used for Figure 4.9).

Table C.5 Summary of cell cycle distribution (%) of the different treatment groups.

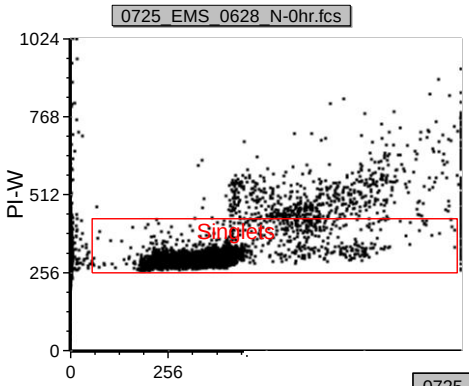
Negative control

Time (hours)	2N	2N to 4N	4N	4N to 8N	8N
0	31.69	54.85	13.47	0	0
26	36.31	38.04	20.22	5.23	0.20
52	52.52	27.31	20.16	0	0

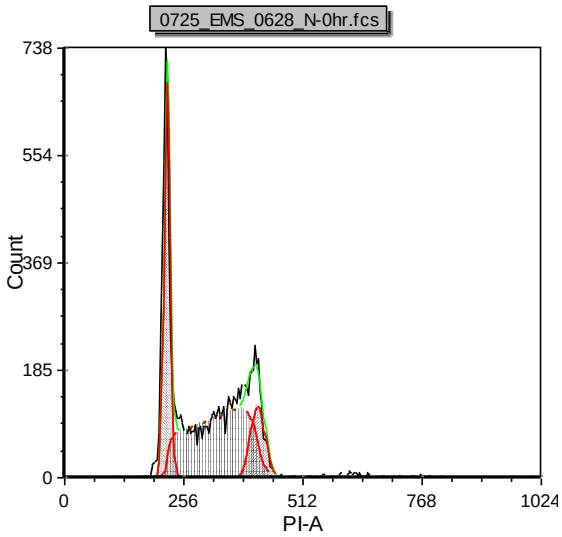
EMS 10 mM

Time (hours)	2N	2N to 4N	4N	4N to 8N	8N
0	27.16	64.55	8.30	0	0
26	14.50	40.03	41.04	3.73	0.71
52	25.78	41.81	32.41	0	0

Negative control, 0 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV S0	14.57	54.45	3.35
SL CL S0	18.71	50.42	1.99
+G2/G1 Fixed	20.52	48.81	2.96
+Aggregates	19.78	49.37	1.97
+S Order = 1	13.47	54.85	2.85
+CVs Fixed	11.19	57.54	2.92



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	221.16	3.00	31.69	416.00	3.74	13.47	54.85	1.88	100.00	0.51

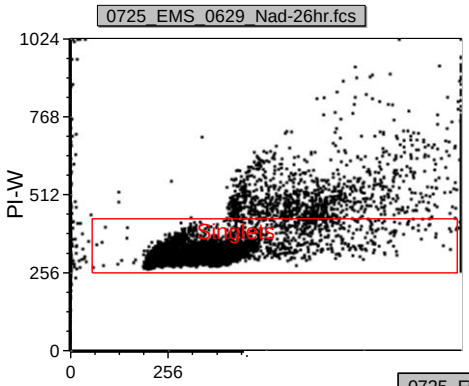
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=54.8, %G2=13.5
The S Phase confidence is good

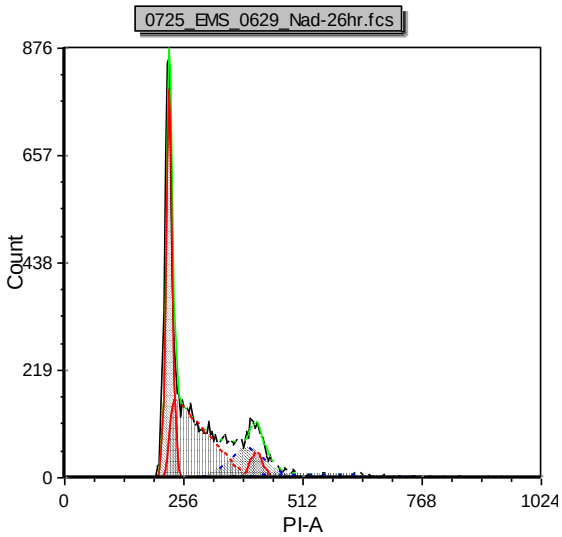
Experiment Statistics

Chi sq: 2.85
BAD: 0.51
Number of cells: 9574.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Negative control, 26 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV S1	6.53	48.43	2.23	27.29	0.99
SL CL S0	4.10	51.65	0.00	43.33	2.16
+G2/G1 Fixed	4.50	49.89	0.00	46.26	2.33
+Aggregates	3.03	52.84	0.00	43.61	2.19
+S Order = 1	5.81	48.19	0.96	24.82	0.97
+CVs Fixed	0.23	57.37	0.80	47.62	1.06



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	225.30	3.12	46.00	413.46	3.12	5.81	48.19	1.84	78.94	n/a	0.14
Aneuploid A	395.73	10.18	74.22	752.07	10.18	0.96	24.82	1.90	21.07	1.76	0.13

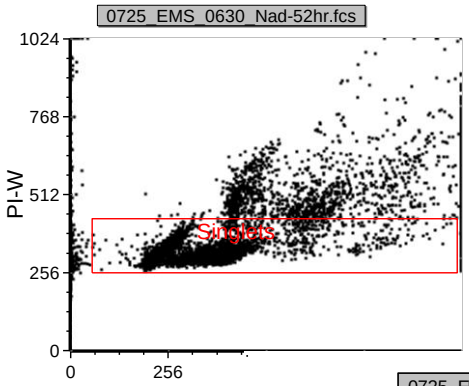
Interpretation

MultiCycle suggestions (a guideline only):
An aneuploid DNA content is observed.
The aneuploid %S=24.8, %G2=0.96
The S Phase confidence is poor
Note: % cells, inter-model error,

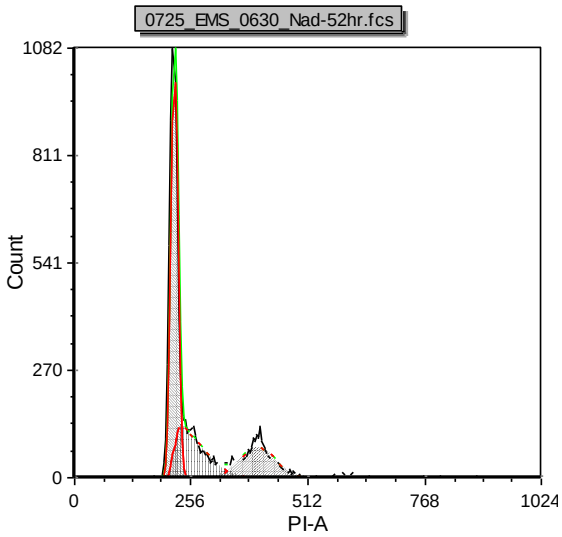
Experiment Statistics

Chi sq: 0.97
Average %S: 43.27
Average %S aneuploid: 24.82
BAD: 0.14
Number of cells: 9808.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

Negative control, 52 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL S0	8.56	29.67	4.42
SL CL S0	7.00	30.31	3.19
+G2/G1 Fixed	5.95	33.19	4.44
+Aggregates	2.77	34.60	3.35
+S Order = 1	20.16	27.31	2.39
+CVs Fixed	11.96	29.94	4.10



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	220.28	3.48	52.52	404.04	9.51	20.16	27.31	1.83	100.00	0.34

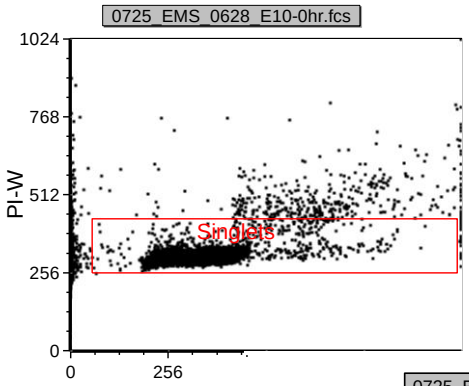
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=27.3, %G2=20.2
The S Phase confidence is good

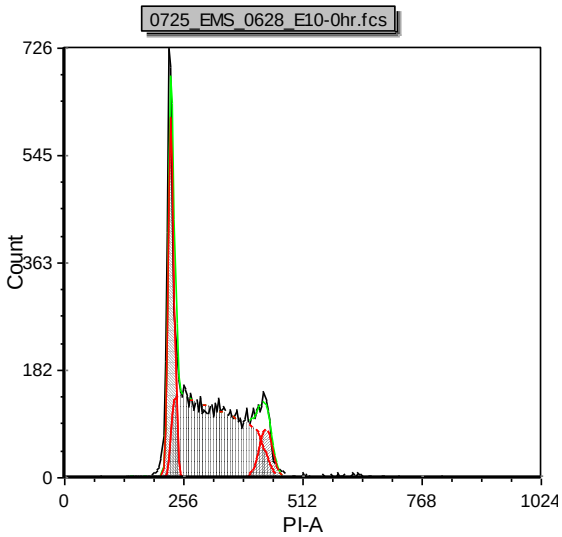
Experiment Statistics

Chi sq: 2.39
BAD: 0.34
Number of cells: 9664.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

EMS 10 mM, 0 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	9.85	61.69	3.98
SL CL S0	4.12	66.69	1.78
+G2/G1 Fixed	10.09	60.21	3.12
+Aggregates	9.47	60.69	2.45
+S Order = 1	8.30	64.55	2.20
+CVs Fixed	7.02	65.53	2.21



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	229.41	2.78	27.16	433.33	3.39	8.30	64.55	1.89	100.00	0.50

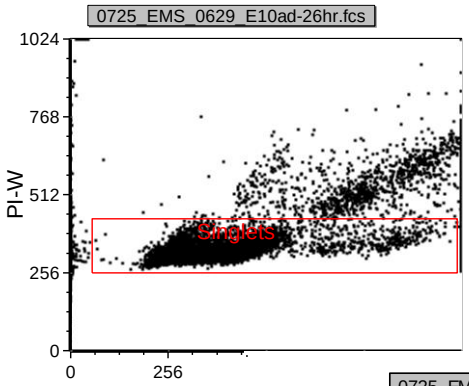
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=64.5, %G2=8.3
The S Phase confidence is poor
Note: intra-model error,

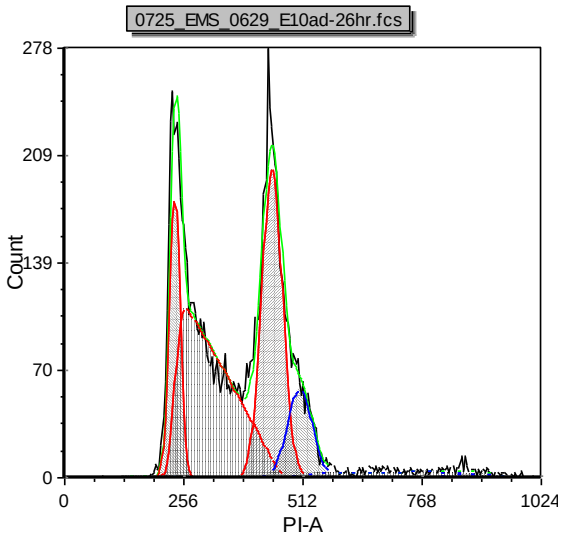
Experiment Statistics

Chi sq: 2.20
BAD: 0.50
Number of cells: 9461.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

EMS 10 mM, 26 hr, +S order=1 model, 2 cycles



Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV G21 S1	28.07	52.72	3.47	14.77	1.62
SL CL S0	31.33	46.73	0.00	29.16	2.19
+G2/G1 Fixed	25.76	50.21	3.06	18.84	2.68
+Aggregates	26.42	49.97	1.45	11.16	2.57
+S Order = 1	35.93	47.03	4.74	25.04	1.31
+CVs Fixed	36.28	46.66	4.79	26.05	1.31



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	237.34	4.98	17.04	447.25	4.98	35.93	47.03	1.88	85.11	n/a	0.42
Aneuploid A	507.04	5.24	70.22	888.56	5.24	4.74	25.04	1.75	14.90	2.14	0.25

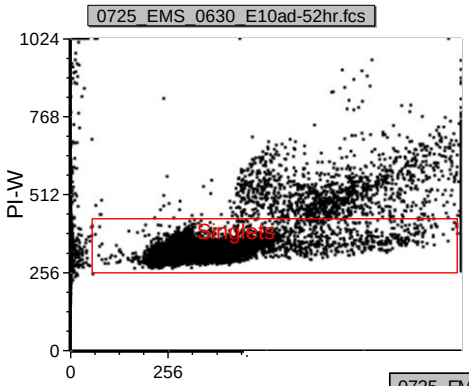
Interpretation

MultiCycle suggestions (a guideline only):
An aneuploid DNA content is observed.
The aneuploid %S=25., %G2=4.74
The S Phase confidence is poor
Note: % cells, intra-model error, inter-model error,

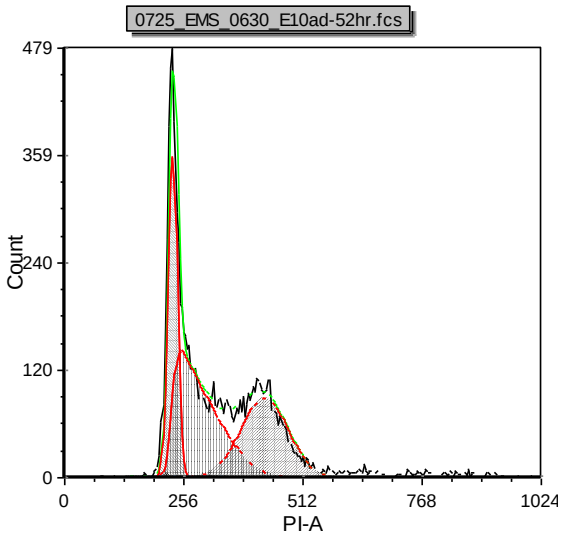
Experiment Statistics

Chi sq: 1.31
Average %S: 43.75
Average %S aneuploid: 25.04
BAD: 0.35
Number of cells: 9420.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

EMS 10 mM, 52 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	34.01	40.98	2.82
SL CL S0	9.09	57.55	1.86
+G2/G1 Fixed	21.65	45.82	3.52
+Aggregates	18.00	47.84	2.14
+S Order = 1	32.41	41.81	2.93
+CVs Fixed	2.85	65.04	3.94



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	232.85	4.33	25.78	430.34	11.90	32.41	41.81	1.85	100.00	0.93

Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=41.8, %G2=32.4
The S Phase confidence is poor
Note: intra-model error, inter-model error,

Experiment Statistics

Chi sq: 2.93
BAD: 0.93
Number of cells: 9318.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Appendix C.7 Plating efficiency of HAN-treated CHO cells. The blank-corrected absorbency values were normalized to the concurrent negative control (raw data used for Figure 4.12).

Table C.6 Cell density of negative control CHO cells measured as the blank-corrected absorbency values normalized to the concurrent negative control (%).

Negative control
98.579
110.942
93.993
98.779
111.939
104.163
112.537
69.068
98.471
87.523
98.256
92.031
108.559
105.339
105.769
104.052
85.218
97.722
96.047
101.600
100.477
106.263
104.355
108.318
102.532
101.024
109.213
115.679
94.343
98.438
99.300
79.472
84.087
96.211
104.547
94.885

Table C.6 (cont.)

105.115
91.286
114.018
109.851
93.572
97.279
88.774
95.993
93.790
101.554
111.847
117.191
93.768
140.604
138.590
97.293
109.380
66.824
71.608
81.933
95.035
96.374
93.473
93.250
97.936
95.035
111.548
117.350
87.791
98.416
102.156
95.424
91.905
112.033
111.307
100.968
95.272
105.102
100.766
108.279
102.162
103.578

Table C.6 (cont.)

100.359
101.990
86.149
99.843
102.763
93.018
92.202
105.210
93.361
111.112
89.691
99.650
102.741
97.182
92.567
106.927
106.519
103.557
88.894
91.112
98.067
104.833
101.459
103.392
104.984
106.576
91.434
106.462
101.042
98.635
90.429
94.390
106.690
108.433
94.750
94.447
103.298
97.650
97.081
103.999
103.563
108.377

Table C.6 (cont.)

93.793
102.603
99.269
108.098
98.598
103.702
104.764
116.890
100.033
100.983
103.795
93.681
86.696
94.631
93.178
105.472
99.679
94.147
102.566
95.786
90.216
96.251
111.954
103.218
92.672
96.920
94.861
104.444
106.896
108.562
101.824
100.963
93.682
93.570
93.233
98.343
88.254
104.033
102.442
110.453
88.835
100.102

Table C.6 (cont.)

	98.886
	96.452
	88.666
	106.316
	107.682
	121.907
number	168.000
Average	100.000
SE	0.739
SD	9.574

Table C.7 Cell density of IAN-treated CHO cells measured as the blank-corrected absorbency values normalized to the concurrent negative control (%).

IAN					
2 µM	3 µM	4 µM	5 µM	6 µM	8 µM
52.517	46.735	39.357		13.235	9.845
91.600	58.101	22.408		7.652	7.253
90.603	81.431	25.997		15.828	8.649
84.821	69.068	33.774		14.033	8.051
81.830	55.110	34.970		16.027	8.649
81.431	46.735	38.759		12.039	26.196
77.642	83.425	60.892		20.613	12.438
61.491	42.149	30.184		19.018	7.652
94.990	32.577	38.759		17.822	11.441
75.249	37.762	19.417		10.643	2.268
93.195	52.119	20.613		19.417	5.259
85.618	36.366	33.574		11.839	6.456
78.041	74.651	29.586		26.396	7.851
84.621	48.330	26.396		11.441	10.244
84.422	62.089	37.562		24.003	21.810
81.630	65.080	28.589		21.211	19.816
95.466	57.472	65.844	24.202	23.343	
89.670	67.132	71.639	24.846	19.265	
89.885	88.167	79.796	26.992	17.118	
88.597	87.953	78.723	31.071	19.909	
94.178	87.953	91.173	25.490	22.055	
76.576	94.822	69.278	23.129	19.050	
87.523	96.968	57.472	29.353	20.767	
82.587	89.241	80.225	56.829	18.406	
87.738	95.680	39.227	33.646	19.265	
98.041	97.183	41.159	21.197	18.406	
103.408	87.523	34.076	50.818	33.002	
91.387	87.953	37.081	23.343	25.275	
99.329	79.152	55.970	24.846	33.432	
97.183	81.299	66.917	20.338	18.621	
107.915	84.948	42.447	29.568	28.280	
103.193	69.708	45.237	24.202	30.641	
88.799	72.205	62.838		39.695	39.017
95.963	81.064	71.124		40.458	46.328
96.620	81.954	73.350		41.115	34.036
101.854	84.285	64.639		42.492	42.047
88.905	90.686	78.584		43.128	35.477
99.566	95.073	71.040		43.637	36.092

Table C.7 (cont.)

	98.591	93.610	77.779		42.153	37.512
	102.427	91.555	82.272		44.781	39.589
	90.071	84.137	63.452		48.066	27.805
	97.086	90.368	68.963		50.291	26.661
	91.682	95.369	65.826		45.926	32.786
	97.743	87.379	87.443		36.198	33.231
	91.449	95.687	80.131		38.190	33.782
	100.562	92.381	71.379		42.386	36.495
	104.843	92.678	77.694		46.773	35.711
	101.240	98.040	80.555		34.291	34.842
number	48	48	48	16	48	32
Average	90.412	76.486	55.295	29.367	27.242	23.290
SE	1.543	2.735	3.066	2.561	1.767	2.439
SD	10.688	18.947	21.238	10.244	12.245	13.798

Table C.8 Cell density of BAN-treated CHO cells measured as the blank-corrected absorbency values normalized to the concurrent negative control (%).

BAN							
2 μM	3 μM	4 μM	6 μM	8 μM	10 μM	12 μM	14 μM
108.998	97.791	73.438	41.541	50.808			
85.722	58.998	38.955	89.170	32.489			
91.110	72.575	44.989	48.006	24.731			
83.351	58.351	54.903	42.834	27.101			
88.739	115.032	51.670	64.386	50.162			
71.282	58.998	79.903	35.075	26.670			
112.877	80.119	110.938	79.041	33.998			
88.739	67.834	52.963	33.998	25.162			
85.291	66.325	109.213	41.756	34.213			
102.963	58.136	102.748	43.481	26.670			
103.179	70.205	48.006	42.188	26.239			
96.498	59.213	118.481	46.498	29.256			
83.136	93.912	86.153	67.188	77.101			
83.351	77.748	77.532	31.843	29.041			
90.894	82.489	76.670	52.748	29.688			
61.153	49.084	63.739	33.567	30.119			
97.158		89.013		30.855	39.380	17.784	
83.708		56.808		51.693	21.383	18.541	
93.370		94.127		66.469	22.141	23.656	
81.435		98.863		42.221	45.063	24.414	
91.475		112.692		83.329	29.150	19.299	
96.590		72.152		58.323	36.159	19.110	
72.910		79.162		80.867	35.022	31.423	
104.736		91.286		63.059	28.960	25.551	
79.541		64.386		40.706	34.454	21.004	
71.205		67.606		77.457	39.190	18.541	
68.932		59.650		33.318	33.318	24.793	
81.435		62.870		76.131	36.349	9.638	
88.634		91.854		31.234	39.380	14.942	
77.646		53.019		36.159	34.833	13.048	
76.131		71.016		53.587	34.833	20.625	
79.541		49.420		37.675	30.855	22.520	
97.803		93.834		57.784		32.312	31.810
95.557		104.454		64.610		32.683	21.299
91.805		103.953		70.738		30.916	20.623
102.012		96.429		73.225		37.546	23.283
93.725		100.856		73.159		39.400	23.676
105.283		111.389		83.300		43.827	36.085

Table C.8 (cont.)

	108.947		108.096		77.390		35.780	22.193
	120.178		107.006		81.163		29.869	25.595
	89.297		88.054		71.480		37.917	31.309
	103.495		96.821		53.968		36.259	36.368
	102.579		97.410		57.850		36.826	21.909
	107.726		84.434		72.134		45.702	25.028
	106.897		99.111		61.034		40.228	26.925
	110.474		101.401		86.571		38.745	20.121
	107.791		103.059		71.524		36.194	30.676
	111.019		105.458		78.066		44.459	24.723
number	48	16	48	16	48	16	32	16
Average	92.423	72.926	83.458	49.582	53.136	33.779	28.861	26.351
SE	1.929	4.402	3.148	4.220	2.981	1.552	1.763	1.330
SD	13.362	17.608	21.813	16.880	20.654	6.209	9.974	5.318

Table C.9 Cell density of CAN-treated CHO cells measured as the blank-corrected absorbency values normalized to the concurrent negative control (%).

CAN						
200 µM	400 µM	600 µM	800 µM	900 µM	1000 µM	1200 µM
	104.344	50.960	33.585		38.370	5.886
	82.184	42.902	21.246		31.571	7.397
	48.946	46.931	28.045		27.794	6.390
	87.472	48.694	27.794		27.038	8.908
	48.190	44.665	30.312		30.312	7.901
	47.183	39.125	26.786		30.815	5.634
	51.464	52.219	38.621		34.341	12.937
	50.960	41.895	26.031		18.728	15.455
	79.163	57.759	17.469		17.218	6.641
	55.744	34.341	17.218		14.448	7.901
	47.938	52.975	15.203		19.736	6.138
	47.938	58.766	35.348		13.189	4.123
	71.357	69.342	33.333		18.980	10.670
	48.190	36.103	31.571		16.966	4.375
	98.552	43.658	40.132		25.779	13.944
	50.708	37.362	22.254		22.254	13.692
98.159	70.042	29.205	12.469	9.121		
85.662	48.173	22.957	12.915	7.782		
95.035	81.423	24.742	15.593	25.188		
89.010	78.075	25.635	12.245	7.336		
93.919	90.126	26.527	28.089	12.469		
82.092	73.835	20.725	16.485	7.782		
91.018	74.505	29.428	27.866	15.593		
106.192	89.456	25.411	19.163	9.791		
51.520	68.926	20.948	15.146	10.460		
47.280	58.215	17.155	13.584	6.220		
61.116	38.801	24.073	9.344	13.808		
79.861	73.612	34.561	16.039	12.022		
64.463	70.042	27.643	15.370	14.700		
86.332	80.753	43.040	17.155	8.452		
117.573	53.752	48.396	17.601	14.477		
98.605	78.298	25.858	16.709	13.138		
68.608	73.360	29.693		11.566		-5.285
99.142	70.104	51.889		15.525		-5.945
97.756	71.798	45.180		19.749		-4.515
97.360	70.038	36.402		15.064		-1.325
76.088	85.547	45.224		18.891		-2.029
91.399	79.189	37.942		20.849		-3.107

Table C.9 (cont.)

	98.086	70.720	36.952		19.287		-1.215
	88.165	70.500	43.464		20.475		-2.271
	54.463	69.202	24.391		13.942		-5.901
	76.308	83.787	29.187		16.999		-2.579
	92.147	68.652	53.099		15.899		-4.185
	85.459	64.406	49.601		9.916		-2.337
	86.559	79.277	31.166		14.162		-3.569
	76.989	78.529	37.172		15.437		-1.347
	90.871	100.110	35.918		15.635		-1.787
	81.983	67.772	47.379		15.591		-2.007
number	32	48	48	32	32	16	32
Average	84.663	69.820	38.305	22.210	13.979	24.221	2.768
SE	2.827	2.248	1.697	1.507	0.792	1.894	1.159
SD	15.990	15.577	11.760	8.524	4.480	7.576	6.557

Table C.10 Cell density of EMS- and AraC-treated CHO cells measured as the blank-corrected absorbency values normalized to the concurrent negative control (%).

EMS		AraC	
5 mM	10 mM	25 µM	50 µM
74.687	28.561	43.240	47.487
77.607	37.683	48.474	51.473
71.253	37.576	51.212	50.132
81.642	34.142	52.628	60.861
72.412	34.184	58.067	55.626
70.717	40.280	53.689	51.473
86.707	36.181	47.487	52.907
86.922	38.434	55.701	50.057
74.988	34.442	44.376	46.145
78.980	30.922	49.051	56.316
79.238	35.687	53.708	56.390
75.954	32.746	50.616	52.236
80.976	37.705	44.096	55.328
86.407	38.220	47.934	49.237
75.117	40.924	48.790	57.564
84.196	37.769	51.268	54.118
76.684	30.149	44.655	36.590
73.872	36.095	49.033	49.312
69.751	39.701	49.834	46.835
77.778	26.371	47.542	43.854
74.001	33.390	48.846	44.022
75.846	38.735	54.490	48.343
81.620	40.817	51.733	45.792
82.629	38.585	48.697	51.268
69.697	34.693	46.722	45.636
81.314	31.604	47.059	47.789
80.707	34.617	52.636	54.414
79.532	37.744	50.035	56.230
74.340	43.600	58.626	51.438
74.264	38.881	57.316	56.380
80.196	40.435	46.235	52.805
80.158	37.422	50.278	47.396
77.410	31.282	49.417	27.706
82.925	33.746	49.118	34.575
80.499	30.277	61.171	54.358
87.814	36.418	61.714	34.968
80.404	37.062	47.414	40.882
80.499	40.909	52.262	47.826

Table C.10 (cont.)

	85.730	41.629	62.800	50.709
	78.661	37.043	51.476	41.069
	70.455	23.910	50.484	32.722
	78.869	25.217	58.794	30.345
	77.694	33.916	56.043	34.406
	95.546	21.219	52.468	31.112
	84.024	31.699	48.425	33.826
	84.365	33.746	56.211	43.690
	83.474	31.756	61.620	45.037
	91.055	29.235	56.043	40.059
number	48	48	48	48
Average	79.367	34.945	51.657	46.849
SE	0.808	0.699	0.709	1.187
SD	5.598	4.842	4.914	8.224

Appendix C.8 Raw data of the DNA strand breaks measured by single cell gel electrophoresis assay. Treated cells were harvested at 0 and 26 hour post-incubation. Values shown are the mean % tail DNA of 50 nuclei of each treatment group (raw data used for Figure 4.13).

Time (hours)	Negative control	CAN 900 µM	BAN 12 µM	IAN 6 µM	AraC 50 µM
0 hour	0.194800	2.21670	23.2395	60.6644	1.54210
26 hour	0.013900	0.010700	0.109200	7.72050	0.045400

APPENDIX D

APPENDICES FOR CHAPTER 5

Summary of contents:

- Thirty nine pages.
- Appendix D.1 Cell cycle profiles of CHO cells treated with various concentrations of HAAs at 26 hour post-incubation.
- Appendix D.2 Cell cycle profiles of CHO cells treated without and with 100, 200, 300, 400 and 500 µM BAA at 26 hour post-incubation.
- AppendixD.3 Cell cycle profiles of CHO cells treated without or with 50 µM IAA at 0, 4, and 8 hour post-incubation.
- Appendix D.4 Plating efficiency of HAA-treated CHO cells
- Appendix D.5 Raw data of the DNA strand breaks measured by single cell gel electrophoresis assay.

Appendix D.1 Cell cycle profiles of CHO cells treated with various concentrations of HAAs at 26 hour post-incubation. DNA contents in individual cells were measured with LSR II flow cytometer and the cell cycle distributions were calculated using the FCS Express software. A summary table, cell cycle distribution, and fitted curves as well as the fitting models are provided (raw data used for Figure 5.1).

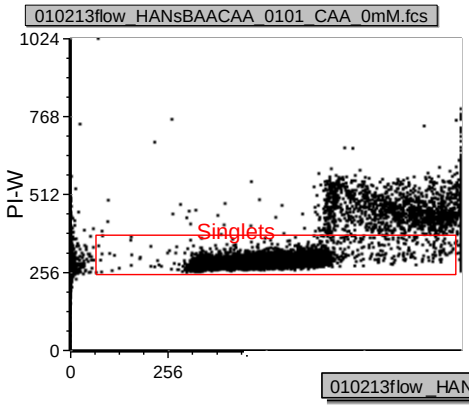
Table D.1 Summary of cell cycle distribution (%) of the different treatment groups.

CAA Concentration (µM)	2N	2N to 4N	4N
0	35.91	59.04	5.05
2	34.76	57.61	7.63
3	37.47	60.22	2.31
4	35.75	58.28	5.98
6	35.47	62.68	1.86
8	30.33	68.05	1.63

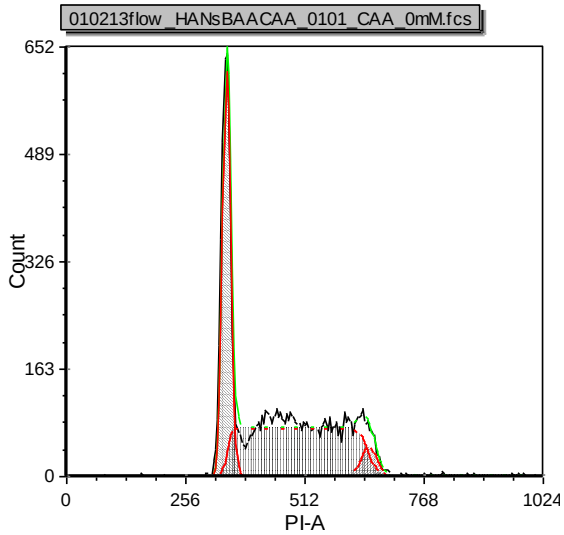
BAA Concentration (µM)	2N	2N to 4N	4N
0	33.21	62.17	4.62
30	30.43	65.77	3.80
60	30.25	65.35	4.41
90	30.45	65.54	4.01
120	29.26	65.60	5.14
150	28.69	61.60	9.71

IAA Concentration (µM)	2N	2N to 4N	4N
0	42.88	53.92	3.21
10	44.57	52.48	2.95
20	44.25	53.11	2.64
30	41.33	55.44	3.23
40	42.42	53.50	4.08
60	40.85	54.54	4.61

CAA 0 mM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	8.34	55.48	2.16
SL CL S0	5.05	59.04	1.55
+G2/G1 Fixed	9.59	54.41	2.16
+Aggregates	8.94	54.85	1.79
+S Order = 1	4.01	59.77	1.91
+CVs Fixed	3.66	60.11	1.90



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	345.43	2.58	35.91	650.98	2.55	5.05	59.04	1.89	100.00	0.29

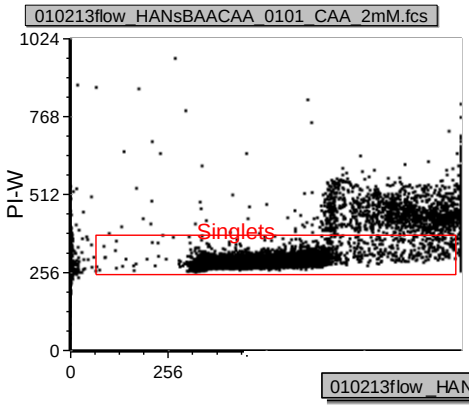
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=59., %G2=5.05
The S Phase confidence is good

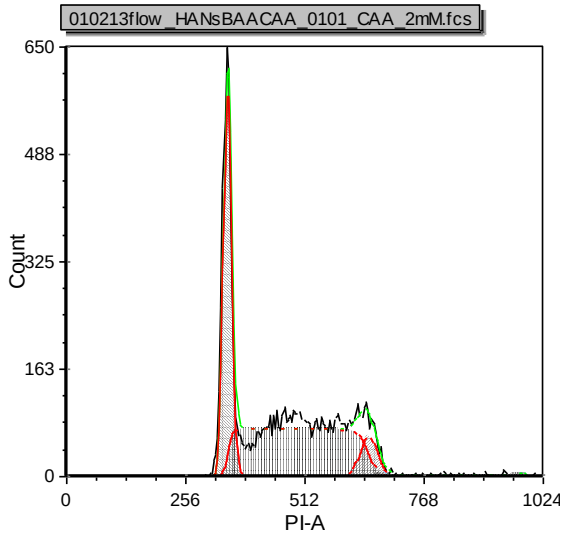
Experiment Statistics

Chi sq: 1.55
BAD: 0.29
Mean Xtra S: 659.37
Number of cells: 10077.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

CAA 2 mM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S1	10.42	54.18	2.66
SL CL S0	7.63	57.61	1.98
+G2/G1 Fixed	12.09	53.18	2.85
+Aggregates	11.12	53.86	2.15
+S Order = 1	3.60	61.00	2.33
+CVs Fixed	1.80	62.68	2.35



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	347.21	2.54	34.76	651.14	2.87	7.63	57.61	1.88	100.00	0.30

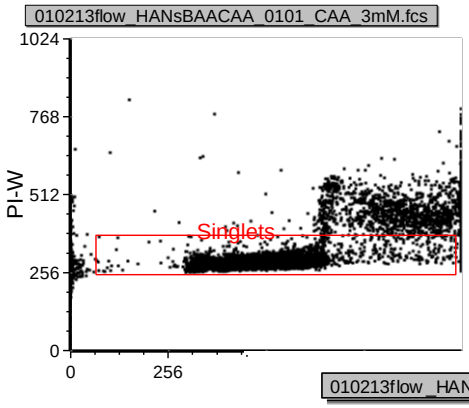
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=57.6, %G2=7.63
The S Phase confidence is good

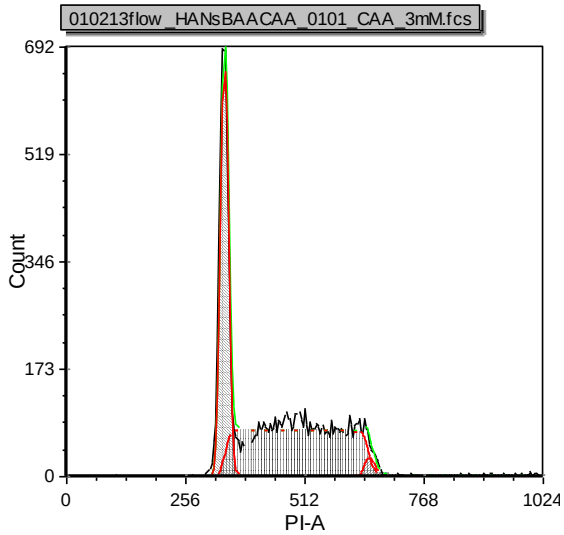
Experiment Statistics

Chi sq: 1.98
BAD: 0.30
Mean Xtra S: 659.37
Number of cells: 10096.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

CAA 3 mM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	5.11	57.53	2.72
SL CL S0	2.31	60.22	2.03
+G2/G1 Fixed	5.11	57.53	2.72
+Aggregates	4.46	57.92	2.10
+S Order = 1	1.07	61.02	2.51
+CVs Fixed	2.25	59.84	2.50



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	340.79	2.55	37.47	652.74	1.66	2.31	60.22	1.92	100.00	0.27

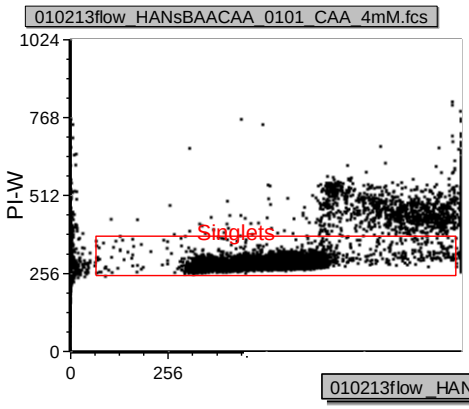
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=60.2, %G2=2.31
The S Phase confidence is good

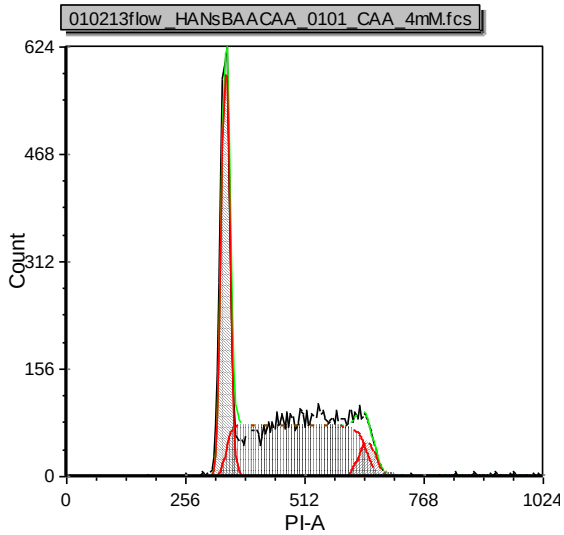
Experiment Statistics

Chi sq: 2.03
BAD: 0.27
Mean Xtra S: 659.37
Number of cells: 10102.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

CAA 4 mM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	5.76	58.59	2.21
SL CL S0	5.98	58.28	1.63
+G2/G1 Fixed	5.75	58.58	2.21
+Aggregates	5.55	58.70	1.63
+S Order = 1	1.42	61.87	1.70
+CVs Fixed	2.22	61.18	1.69



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	343.18	2.67	35.75	647.26	2.71	5.98	58.28	1.89	100.00	0.35

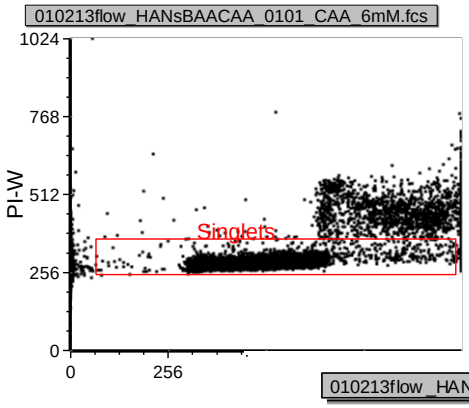
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=58.3, %G2=5.98
The S Phase confidence is good

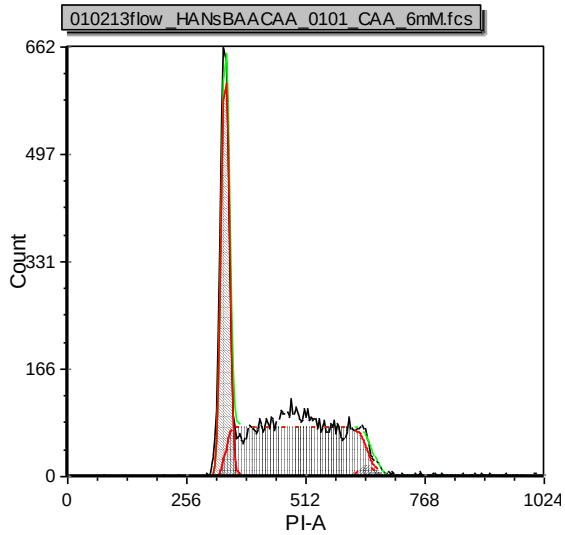
Experiment Statistics

Chi sq: 1.63
BAD: 0.35
Mean Xtra S: 659.37
Number of cells: 10086.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

CAA 6 mM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	3.59	61.22	2.41
SL CL S0	1.86	62.68	1.75
+G2/G1 Fixed	3.59	61.21	2.41
+Aggregates	2.98	61.54	1.76
+S Order = 1	1.71	63.12	2.41
+CVs Fixed	1.25	63.42	2.45



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	340.20	2.57	35.47	647.60	2.74	1.86	62.68	1.90	100.00	0.47

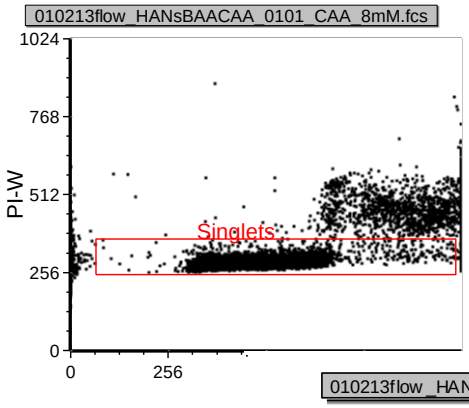
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=62.7, %G2=1.86
The S Phase confidence is good

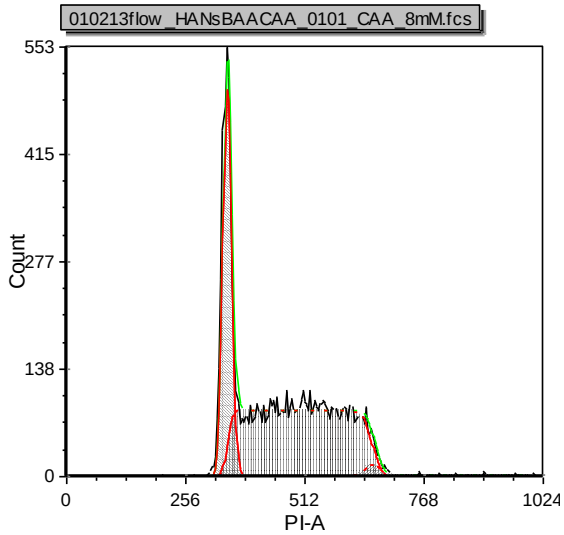
Experiment Statistics

Chi sq: 1.75
BAD: 0.47
Mean Xtra S: 659.37
Number of cells: 10045.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

CAA 8 mM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	2.13	67.53	1.60
SL CL S0	1.63	68.05	1.08
+G2/G1 Fixed	2.13	67.52	1.60
+Aggregates	2.19	67.24	1.06
+S Order = 1	1.55	67.97	1.59
+CVs Fixed	1.71	68.02	1.67



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	346.68	2.69	30.33	658.67	2.43	1.63	68.05	1.90	100.00	0.34

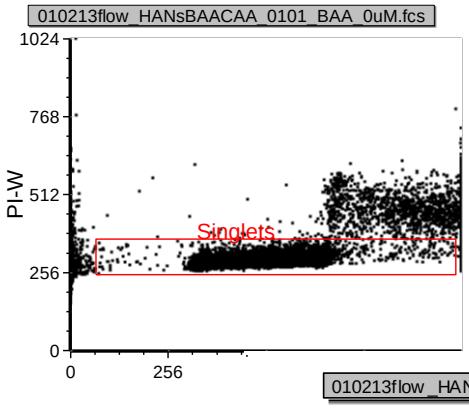
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=68., %G2=1.62
The S Phase confidence is good

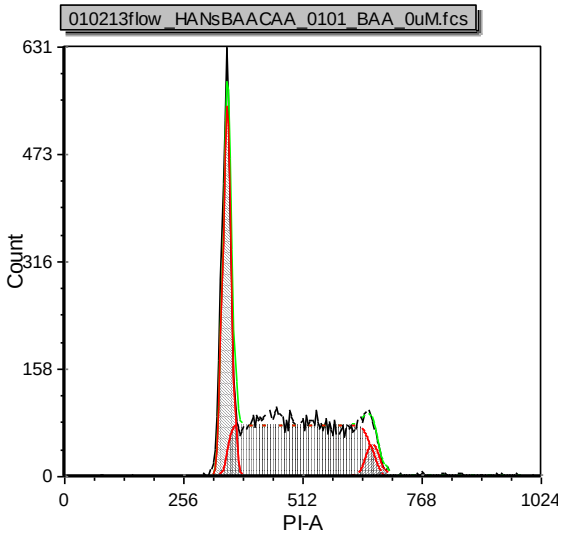
Experiment Statistics

Chi sq: 1.08
BAD: 0.34
Mean Xtra S: 659.37
Number of cells: 10031.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAA 0 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	5.66	61.26	1.81
SL CL S0	4.62	62.17	1.28
+G2/G1 Fixed	5.66	61.26	1.81
+Aggregates	5.13	61.65	1.28
+S Order = 1	5.86	61.40	1.79
+CVs Fixed	5.63	61.62	1.79



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	350.18	2.65	33.21	663.68	2.32	4.62	62.17	1.90	100.00	0.43

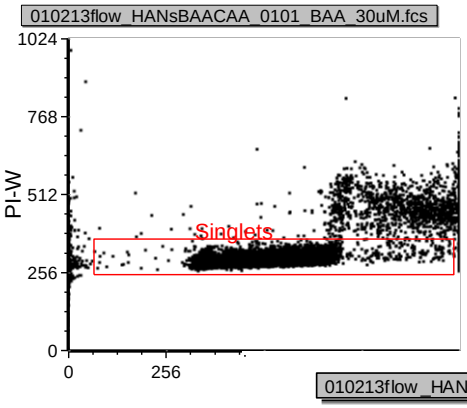
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=62.2, %G2=4.62
The S Phase confidence is good

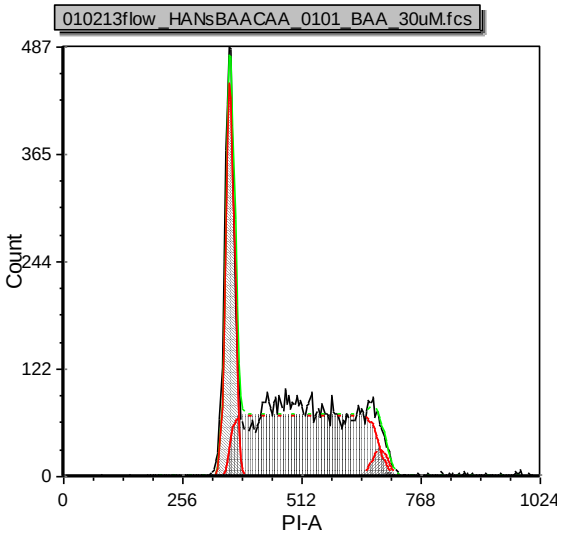
Experiment Statistics

Chi sq: 1.28
BAD: 0.43
Mean Xtra S: 659.37
Number of cells: 10042.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAA 30 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	5.29	64.38	1.91
SL CL S0	3.80	65.77	1.47
+G2/G1 Fixed	5.29	64.38	1.90
+Aggregates	4.77	64.76	1.48
+S Order = 1	3.90	65.74	1.90
+CVs Fixed	3.78	65.83	1.89



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	358.53	2.60	30.43	681.23	2.39	3.80	65.77	1.90	100.00	0.60

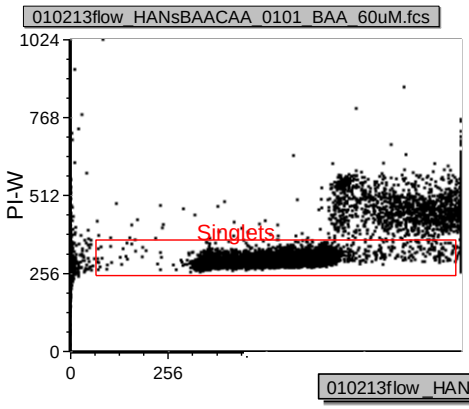
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=65.8, %G2=3.8
The S Phase confidence is good

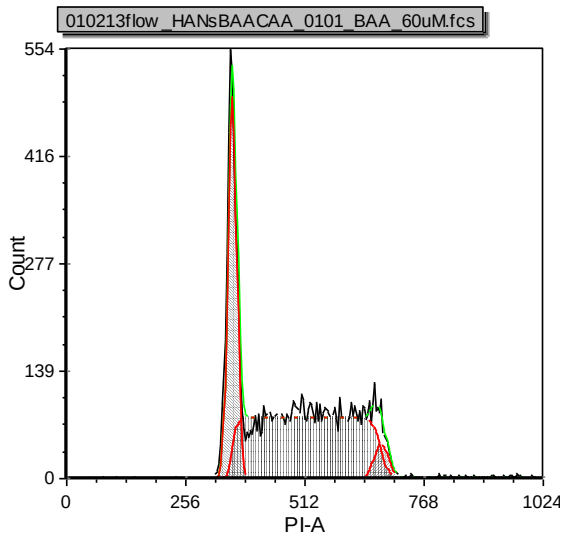
Experiment Statistics

Chi sq: 1.47
BAD: 0.60
Mean Xtra S: 659.37
Number of cells: 9052.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAA 60 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	5.53	64.29	2.00
SL CL S0	4.41	65.35	1.58
+G2/G1 Fixed	5.53	64.29	2.00
+Aggregates	5.11	64.59	1.58
+S Order = 1	2.77	66.39	1.85
+CVs Fixed	3.82	65.42	1.85



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	357.16	2.63	30.25	677.54	2.17	4.41	65.35	1.90	100.00	0.33

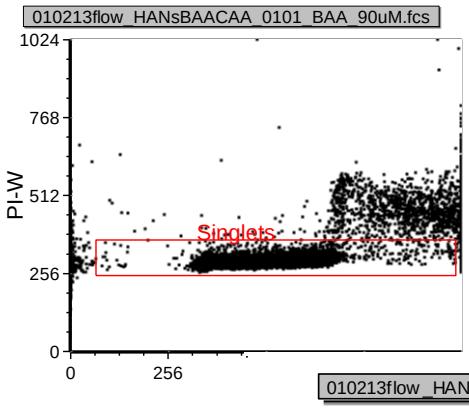
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=65.3, %G2=4.41
The S Phase confidence is good

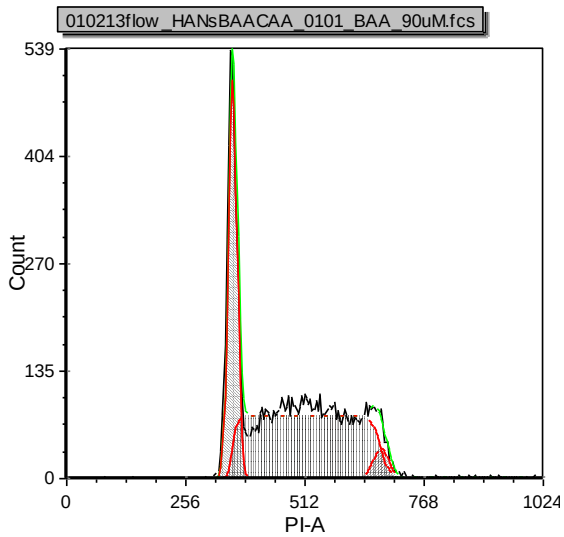
Experiment Statistics

Chi sq: 1.58
BAD: 0.33
Mean Xtra S: 659.37
Number of cells: 10048.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAA 90 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	4.60	65.06	1.85
SL CL S0	4.01	65.54	1.40
+G2/G1 Fixed	4.60	65.05	1.85
+Aggregates	4.23	65.31	1.40
+S Order = 1	2.80	66.32	1.78
+CVs Fixed	2.84	66.32	1.77



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	357.82	2.60	30.45	677.21	2.36	4.01	65.54	1.89	100.00	0.31

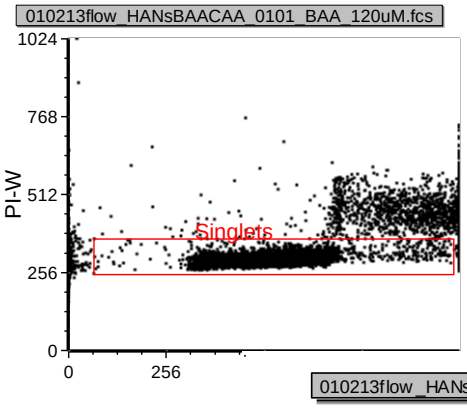
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=65.5, %G2=4.01
The S Phase confidence is good

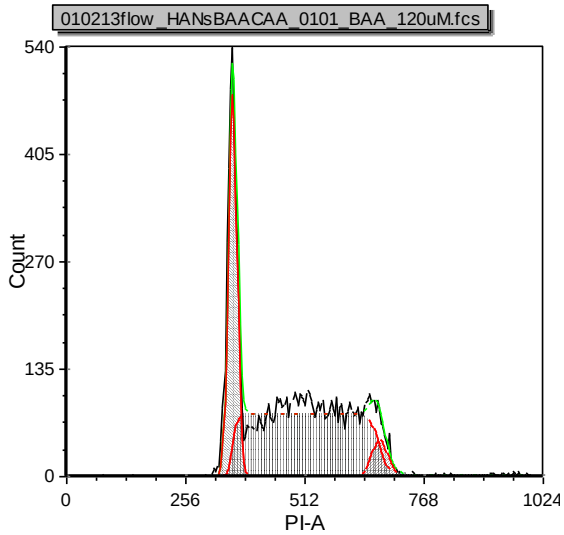
Experiment Statistics

Chi sq: 1.40
BAD: 0.31
Mean Xtra S: 659.37
Number of cells: 10053.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAA 120 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	4.98	65.87	2.55
SL CL S0	5.14	65.60	2.01
+G2/G1 Fixed	4.98	65.87	2.55
+Aggregates	4.65	66.11	2.01
+S Order = 1	2.74	67.27	2.33
+CVs Fixed	2.82	67.23	2.32



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	357.84	2.58	29.26	674.76	2.48	5.14	65.60	1.89	100.00	0.36

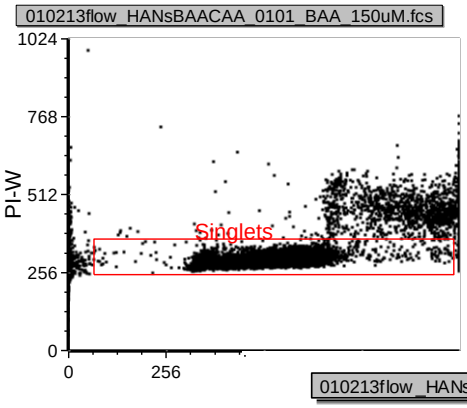
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=65.6, %G2=5.14
The S Phase confidence is good

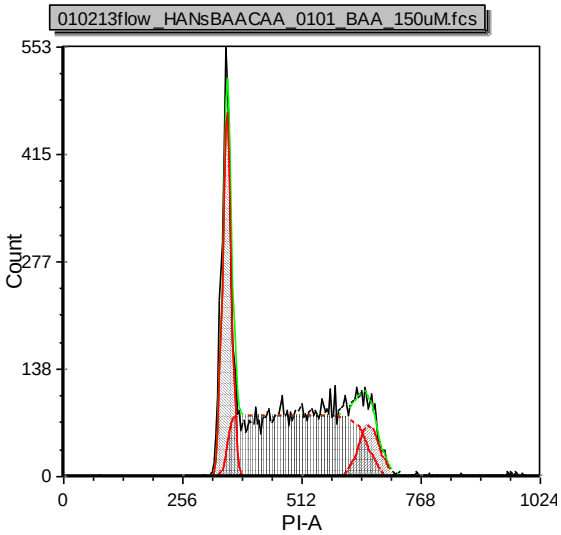
Experiment Statistics

Chi sq: 2.01
BAD: 0.36
Mean Xtra S: 659.37
Number of cells: 10055.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAA 150 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	6.94	64.45	1.73
SL CL S0	9.71	61.60	1.23
+G2/G1 Fixed	6.94	64.44	1.73
+Aggregates	6.60	64.73	1.29
+S Order = 1	6.82	63.80	1.47
+CVs Fixed	4.19	66.23	1.49



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	352.10	2.66	28.69	657.38	3.45	9.71	61.60	1.87	100.00	0.33

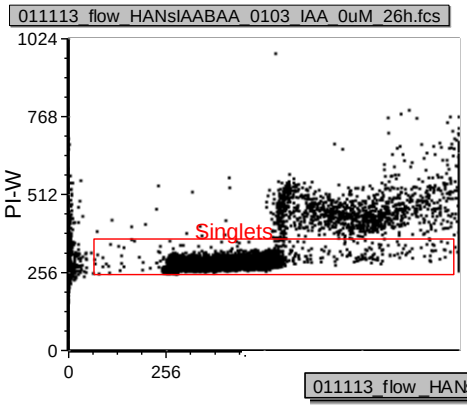
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=61.6, %G2=9.71
The S Phase confidence is good

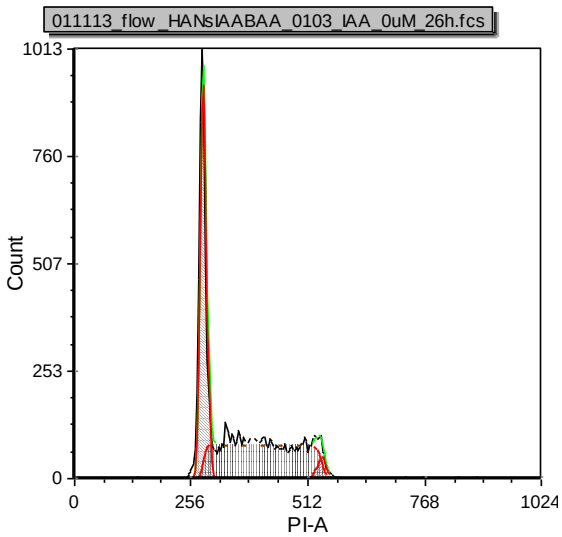
Experiment Statistics

Chi sq: 1.23
BAD: 0.33
Mean Xtra S: 659.37
Number of cells: 10042.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAA 0 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	7.60	49.10	2.13
SL CL S0	3.21	53.92	1.33
+G2/G1 Fixed	7.60	49.11	2.13
+Aggregates	7.07	49.50	1.94
+S Order = 1	4.26	53.28	1.64
+CVs Fixed	4.55	53.01	1.64



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	283.86	2.44	42.88	543.11	1.74	3.21	53.92	1.91	100.00	0.29

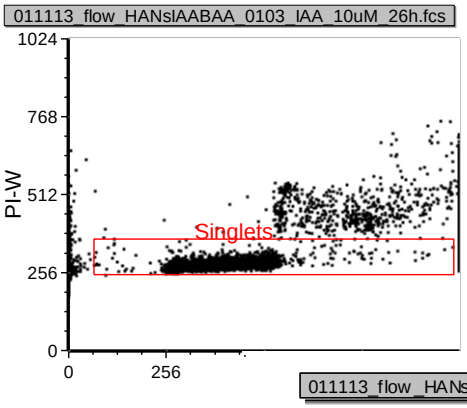
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=53.9, %G2=3.21
The S Phase confidence is good

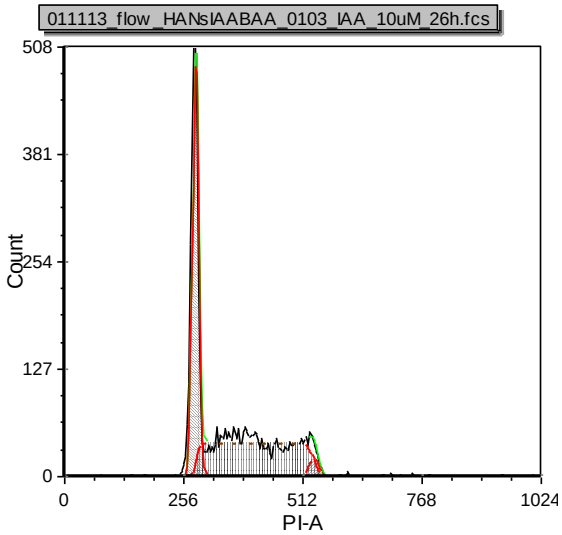
Experiment Statistics

Chi sq: 1.33
BAD: 0.29
Mean Xtra S: 659.37
Number of cells: 9947.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAA 10 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	7.04	48.23	1.37
SL CL S0	2.95	52.48	1.10
+G2/G1 Fixed	7.04	48.23	1.37
+Aggregates	6.51	48.55	1.24
+S Order = 1	3.95	51.88	1.22
+CVs Fixed	4.01	51.78	1.22



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	282.85	2.34	44.57	537.87	1.92	2.95	52.48	1.90	100.00	0.60

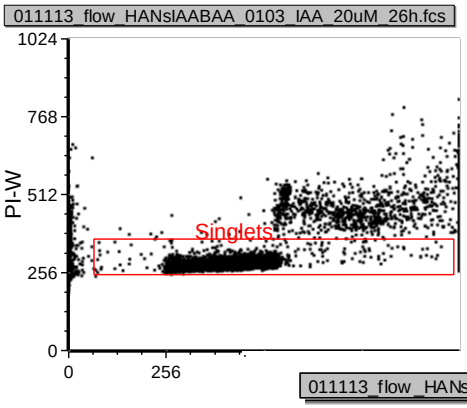
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=52.5, %G2=2.95
The S Phase confidence is good

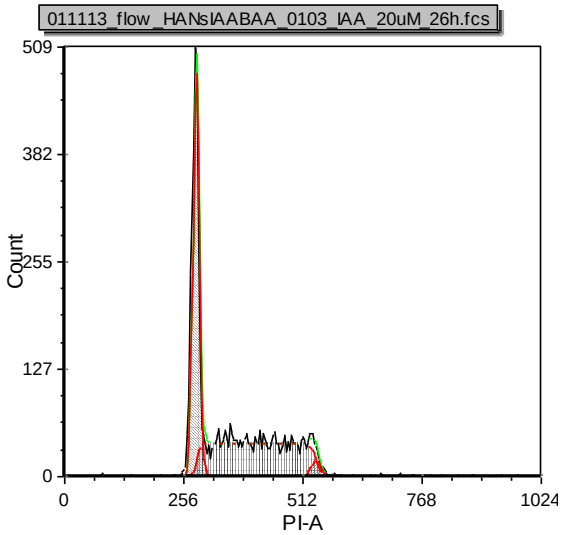
Experiment Statistics

Chi sq: 1.10
BAD: 0.60
Mean Xtra S: 659.37
Number of cells: 4983.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAA 20 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	6.60	48.89	1.32
SL CL S0	2.64	53.11	0.99
+G2/G1 Fixed	6.57	48.91	1.32
+Aggregates	6.43	48.99	1.21
+S Order = 1	2.79	52.98	1.15
+CVs Fixed	3.50	52.33	1.16



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	284.07	2.41	44.25	541.90	1.82	2.64	53.11	1.91	100.00	0.47

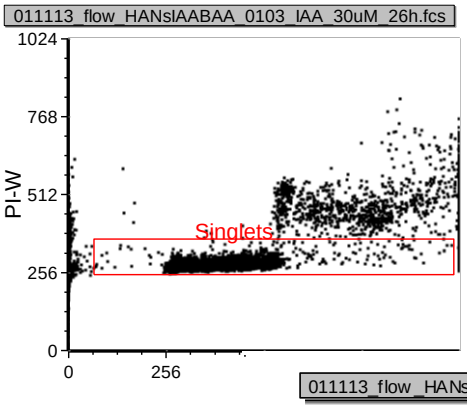
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=53.1, %G2=2.64
The S Phase confidence is good

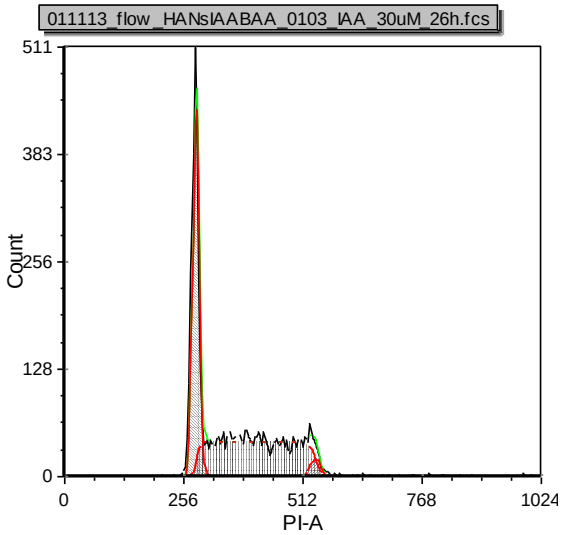
Experiment Statistics

Chi sq: 0.99
BAD: 0.47
Mean Xtra S: 659.37
Number of cells: 4965.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAA 30 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	7.33	50.97	1.24
SL CL S0	3.23	55.44	0.86
+G2/G1 Fixed	7.33	50.96	1.22
+Aggregates	7.09	51.09	1.10
+S Order = 1	4.32	54.73	1.01
+CVs Fixed	4.46	54.61	1.01



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	283.51	2.45	41.33	540.86	2.00	3.23	55.44	1.91	100.00	0.37

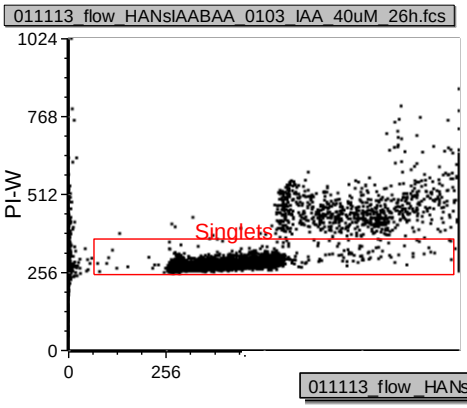
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=55.4, %G2=3.23
The S Phase confidence is good

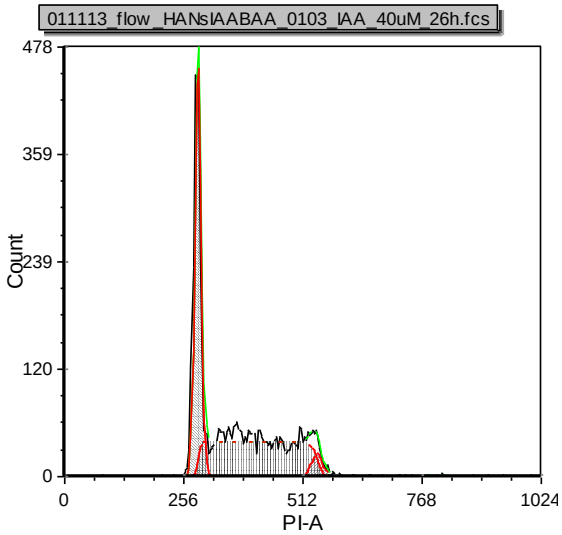
Experiment Statistics

Chi sq: 0.86
BAD: 0.37
Mean Xtra S: 659.37
Number of cells: 4963.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAA 40 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	7.79	49.66	1.23
SL CL S0	4.08	53.50	0.94
+G2/G1 Fixed	7.78	49.67	1.23
+Aggregates	7.19	50.08	1.06
+S Order = 1	5.79	52.38	1.09
+CVs Fixed	4.98	53.08	1.09



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	287.66	2.38	42.42	543.71	2.09	4.08	53.50	1.89	100.00	0.28

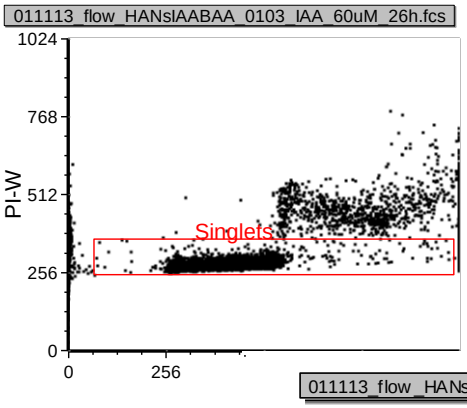
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=53.5, %G2=4.08
The S Phase confidence is good

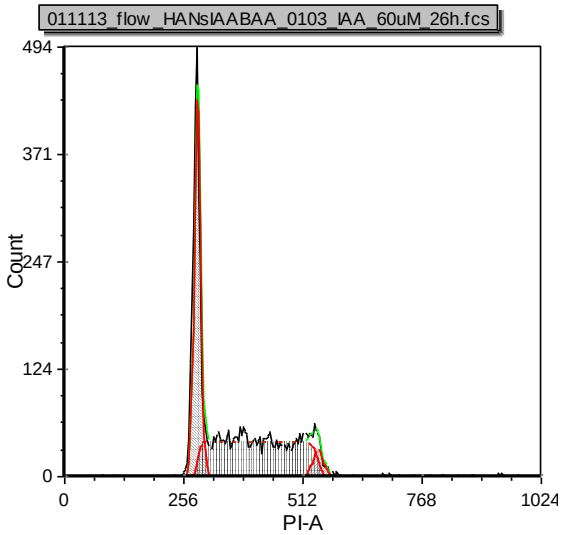
Experiment Statistics

Chi sq: 0.94
BAD: 0.28
Mean Xtra S: 659.37
Number of cells: 4958.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAA 60 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	9.04	49.63	1.17
SL CL S0	4.61	54.54	0.82
+G2/G1 Fixed	9.04	49.63	1.17
+Aggregates	8.79	49.84	1.07
+S Order = 1	5.91	53.55	0.95
+CVs Fixed	5.90	53.52	0.95



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	286.62	2.42	40.85	545.84	1.97	4.61	54.54	1.90	100.00	0.47

Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=54.5, %G2=4.61
The S Phase confidence is good

Experiment Statistics

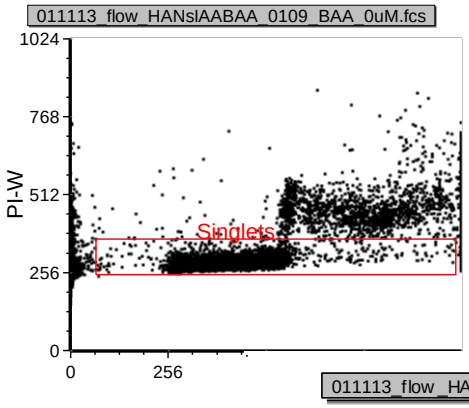
Chi sq: 0.82
BAD: 0.47
Mean Xtra S: 659.37
Number of cells: 4958.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Appendix D.2 Cell cycle profiles of CHO cells treated without and with 100, 200, 300, 400 and 500 µM BAA at 26 hour post-incubation. DNA contents in individual cells were measured with LSR II flow cytometer and the cell cycle distributions were calculated using the FCS Express software. A summary table, cell cycle distribution, and fitted curves as well as the fitting models are provided (raw data used for Figure 5.2).

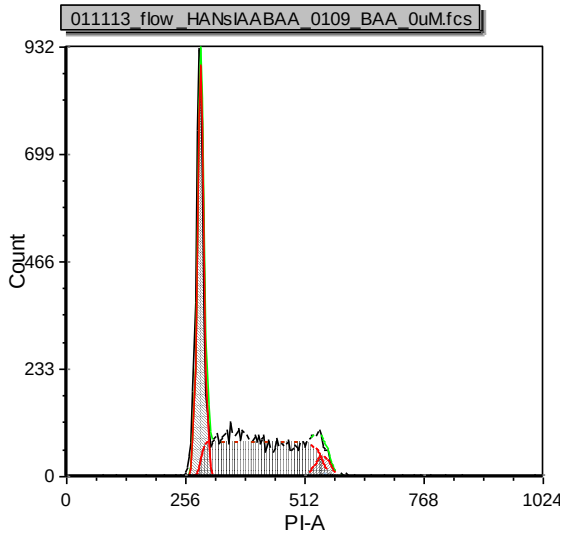
Table D.2 Summary of cell cycle distribution (%) of the different treatment groups.

BAA Concentration (µM)	2N	2N to 4N	4N
0	43.72	51.58	4.70
100	40.41	56.45	3.14
200	35.22	54.24	10.54
300	33.33	56.88	9.79
400	31.87	50.75	17.38
500	31.78	50.31	17.91

BAA 0 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	8.67	47.21	2.33
SL CL S0	4.70	51.58	1.43
+G2/G1 Fixed	8.67	47.21	2.33
+Aggregates	8.32	47.40	1.95
+S Order = 1	7.23	50.34	1.57
+CVs Fixed	5.81	51.47	1.63



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	288.99	2.55	43.72	550.82	2.70	4.70	51.58	1.91	100.00	0.56

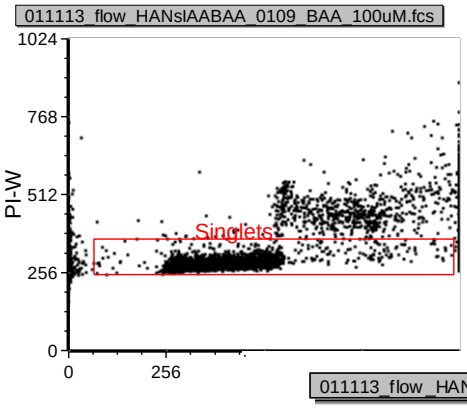
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=51.6, %G2=4.7
The S Phase confidence is good

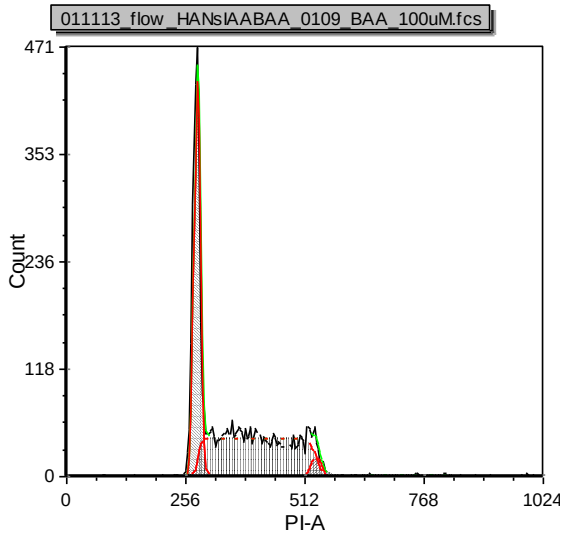
Experiment Statistics

Chi sq: 1.43
BAD: 0.56
Mean Xtra S: 659.37
Number of cells: 9910.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAA 100 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	7.03	52.14	1.45
SL CL S0	3.14	56.45	0.94
+G2/G1 Fixed	7.00	52.18	1.45
+Aggregates	6.20	52.79	1.29
+S Order = 1	5.47	55.39	1.08
+CVs Fixed	4.76	55.96	1.09



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	282.00	2.44	40.41	538.19	2.10	3.14	56.45	1.91	100.00	0.48

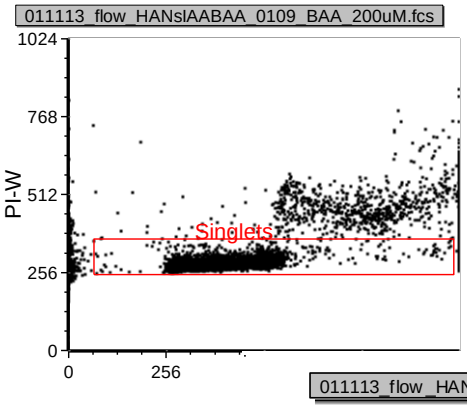
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=56.5, %G2=3.14
The S Phase confidence is good

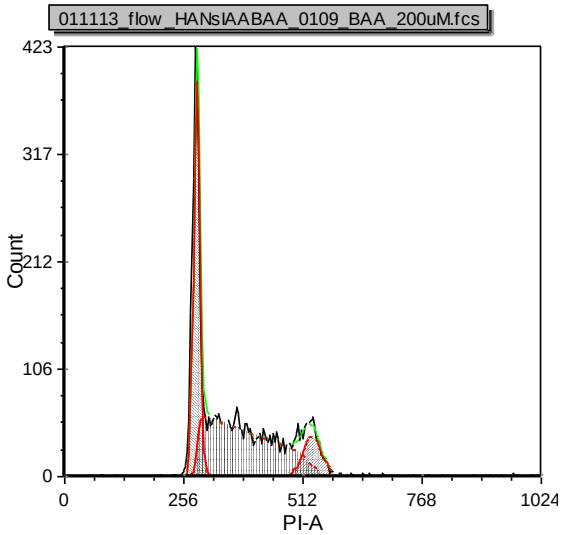
Experiment Statistics

Chi sq: 0.94
BAD: 0.48
Mean Xtra S: 659.37
Number of cells: 4958.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAA 200 µM, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	9.02	52.43	1.54
SL CL S0	5.28	56.63	1.16
+G2/G1 Fixed	9.00	52.46	1.54
+Aggregates	8.89	52.52	1.40
+S Order = 1	10.54	54.24	0.83
+CVs Fixed	7.15	56.79	0.99



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	285.18	2.41	35.22	533.35	3.82	10.54	54.24	1.87	100.00	0.40

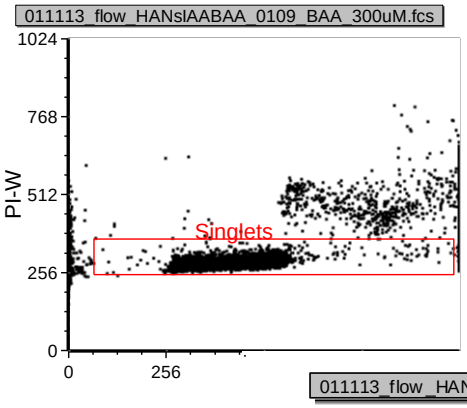
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=54.2, %G2=10.5
The S Phase confidence is good

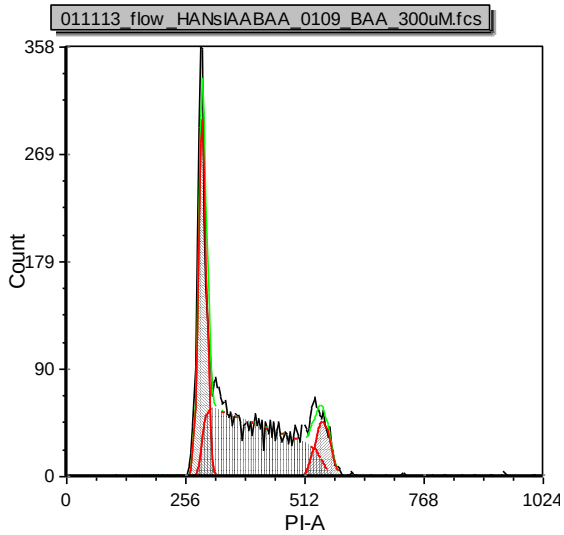
Experiment Statistics

Chi sq: 0.83
BAD: 0.40
Mean Xtra S: 659.37
Number of cells: 4966.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAA 300 µM, 26 hr, +CVs fixed model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	10.54	52.83	1.79
SL CL S0	6.69	57.38	1.40
+G2/G1 Fixed	10.40	53.18	1.78
+Aggregates	10.44	52.94	1.67
+S Order = 1	12.89	54.80	1.44
+CVs Fixed	9.79	56.88	1.11



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	292.39	2.92	33.33	551.19	2.92	9.79	56.88	1.89	100.00	0.65

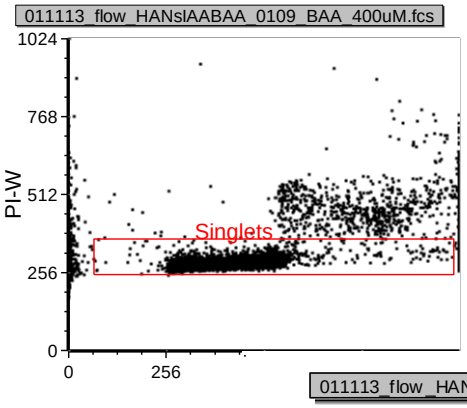
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=56.9, %G2=9.79
The S Phase confidence is good

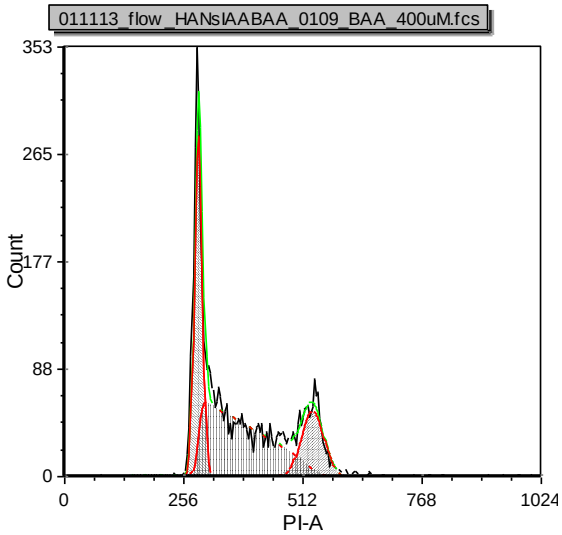
Experiment Statistics

Chi sq: 1.11
BAD: 0.65
Mean Xtra S: 659.37
Number of cells: 5065.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAA 400 µM, 26 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	12.92	50.32	2.08
SL CL S0	9.75	53.92	1.61
+G2/G1 Fixed	12.91	50.35	2.08
+Aggregates	12.21	50.93	1.75
+S Order = 1	17.38	50.75	1.35
+CVs Fixed	13.29	53.38	1.51



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	288.35	2.90	31.87	534.34	4.50	17.38	50.75	1.85	100.00	0.60

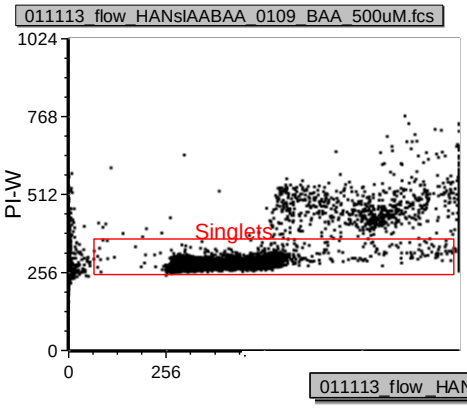
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=50.8, %G2=17.4
The S Phase confidence is good

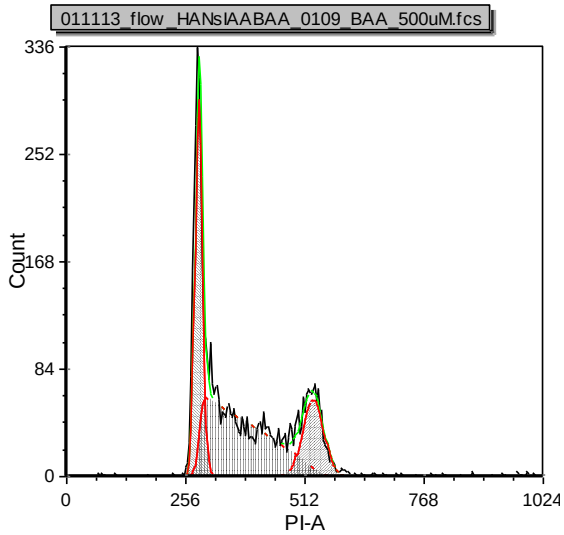
Experiment Statistics

Chi sq: 1.35
BAD: 0.60
Mean Xtra S: 659.37
Number of cells: 4944.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

BAA 500 µM, 26 hr, sliced background model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL CV G21 S0	14.15	49.00	2.22
SL CL S0	11.13	52.60	1.70
+G2/G1 Fixed	14.15	49.00	2.22
+Aggregates	13.92	49.13	1.93
+S Order = 1	17.91	50.31	1.28
+CVs Fixed	14.36	52.46	1.49



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	285.09	2.83	31.78	531.33	4.23	17.91	50.31	1.86	100.00	0.29

Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=50.3, %G2=17.9
The S Phase confidence is good

Experiment Statistics

Chi sq: 1.28
BAD: 0.29
Mean Xtra S: 659.37
Number of cells: 4976.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Appendix D.3 Cell cycle profiles of CHO cells treated without or with 50 µM IAA at 0, 4, and 8 hour post-incubation. DNA contents in individual cells were measured with LSR II flow cytometer and the cell cycle distributions were calculated using the FCS Express software. A summary table, cell cycle distribution, and fitted curves as well as the fitting models are provided (raw data used for Figure 5.3).

Table D.3 Summary of cell cycle distribution (%) of the different treatment groups.

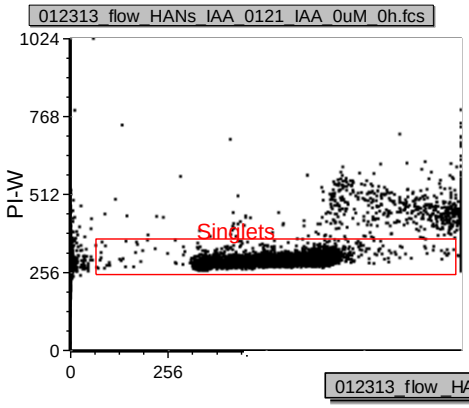
Negative control

Time (hours)	2N	2N to 4N	4N
0	29.20	53.74	17.06
4	47.78	12.34	39.88
8	45.41	47.49	7.10

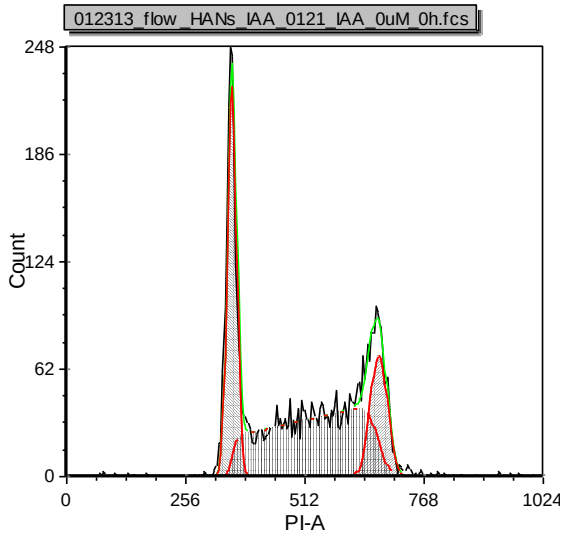
IAA 50 µM

Time (hours)	2N	2N to 4N	4N
0	26.73	57.77	15.50
4	44.34	15.34	40.32
8	44.74	45.87	9.40

Negative control, 0 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL S0	20.53	51.16	1.15
SL CL S0	20.24	51.37	1.09
+G2/G1 Fixed	1.18	68.64	3.35
+Aggregates	0.84	68.88	3.35
+S Order = 1	17.06	53.74	0.98
+CVs Fixed	17.05	53.74	0.97



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	356.39	2.75	29.20	673.00	2.75	17.06	53.74	1.89	100.00	0.57

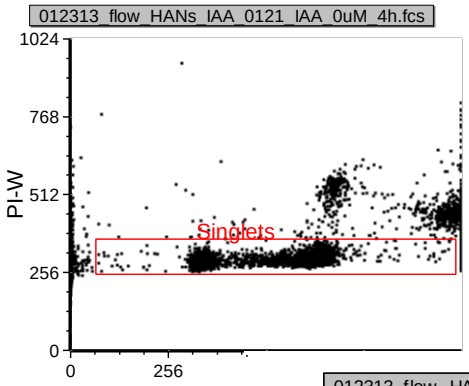
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=53.7, %G2=17.1
The S Phase confidence is fair
Note: inter-model error,

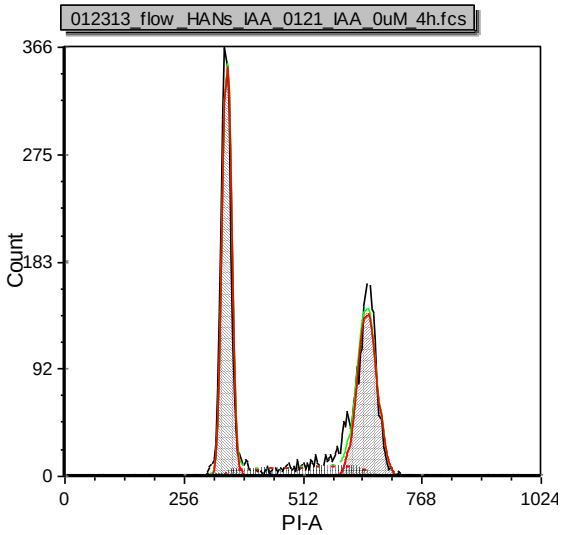
Experiment Statistics

Chi sq: 0.98
BAD: 0.57
Mean Xtra S: 659.37
Number of cells: 4997.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Negative control, 4 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL S0	41.02	11.05	1.78
SL CL S0	40.82	11.14	1.76
+G2/G1 Fixed	5.63	23.90	9.87
+Aggregates	7.24	23.26	10.07
+S Order = 1	39.88	12.34	1.64
+CVs Fixed	39.46	12.64	1.69



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	349.65	2.90	47.78	652.18	3.25	39.88	12.34	1.87	100.00	0.77

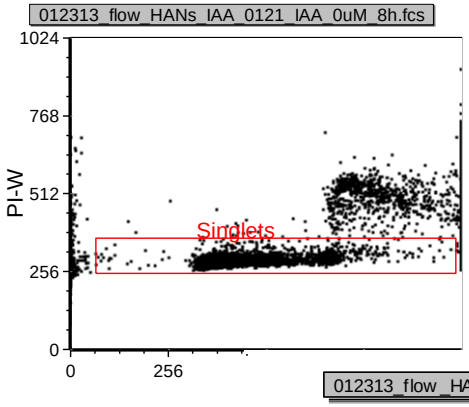
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=12.3, %G2=39.9
The S Phase confidence is fair
Note: inter-model error,

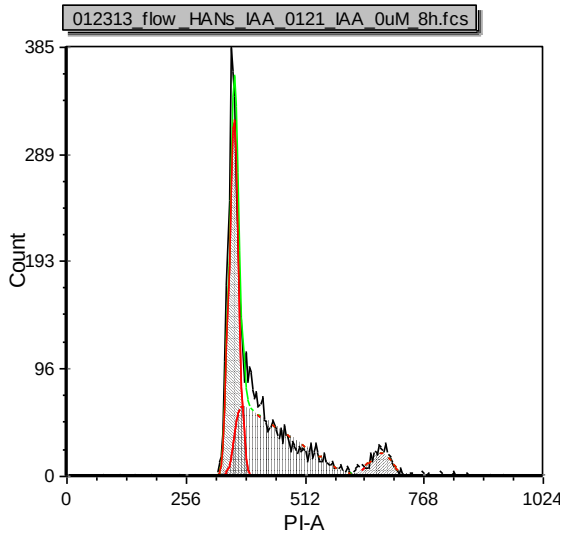
Experiment Statistics

Chi sq: 1.64
BAD: 0.77
Mean Xtra S: 659.37
Number of cells: 4976.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Negative control, 8 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL S0	0.00	33.89	6.39
SL CL S0	0.00	33.98	6.46
+G2/G1 Fixed	0.00	33.81	6.35
+Aggregates	0.00	33.98	6.42
+S Order = 1	7.10	47.49	1.59
+CVs Fixed	7.07	46.66	1.63



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	360.10	2.89	45.41	676.20	3.61	7.10	47.49	1.88	100.00	0.26

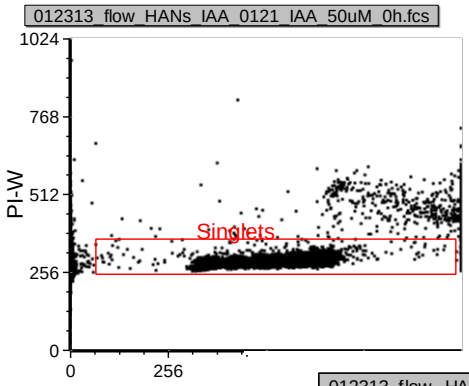
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=47.5, %G2=7.1
The S Phase confidence is poor
Note: intra-model error,

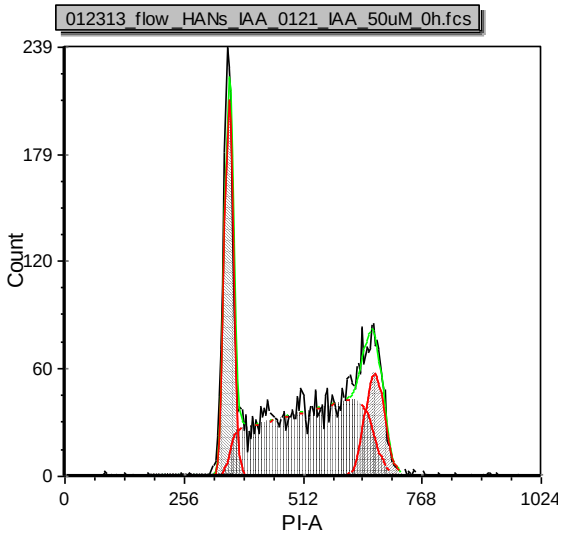
Experiment Statistics

Chi sq: 1.59
BAD: 0.26
Mean Xtra S: 659.37
Number of cells: 4982.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAA 50 µM, 0 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL S0	20.32	53.61	1.17
SL CL S0	20.15	53.73	1.13
+G2/G1 Fixed	1.21	71.62	2.84
+Aggregates	0.68	71.95	3.04
+S Order = 1	15.50	57.77	0.96
+CVs Fixed	14.12	59.36	0.99



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	353.96	2.76	26.73	668.26	3.11	15.50	57.77	1.89	100.00	0.74

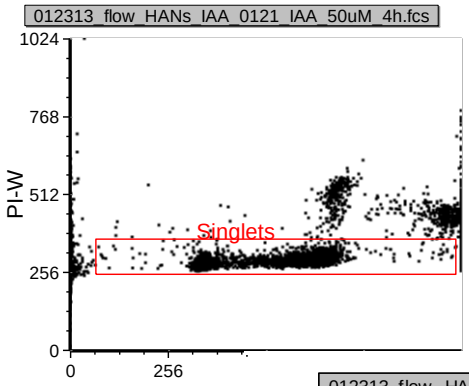
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=57.8, %G2=15.5
The S Phase confidence is good

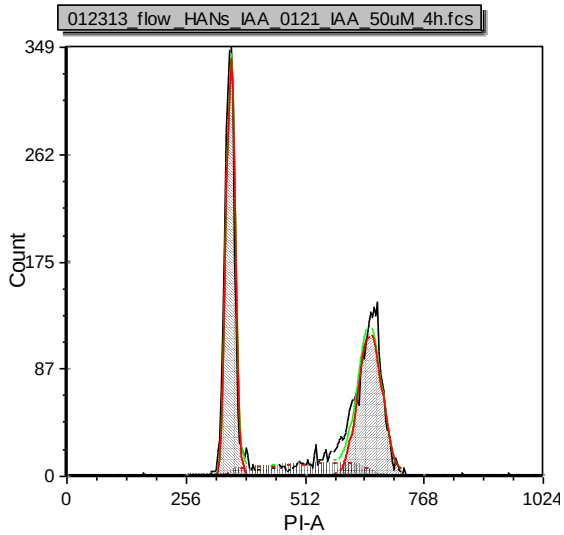
Experiment Statistics

Chi sq: 0.96
BAD: 0.74
Mean Xtra S: 659.37
Number of cells: 4994.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAA 50 µM, 4 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL S0	42.03	13.21	1.64
SL CL S0	41.90	13.23	1.63
+G2/G1 Fixed	6.34	29.56	9.27
+Aggregates	5.65	29.76	9.26
+S Order = 1	40.32	15.34	1.50
+CVs Fixed	38.04	16.79	2.02



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	353.53	2.76	44.34	654.22	3.97	40.32	15.34	1.85	100.00	0.90

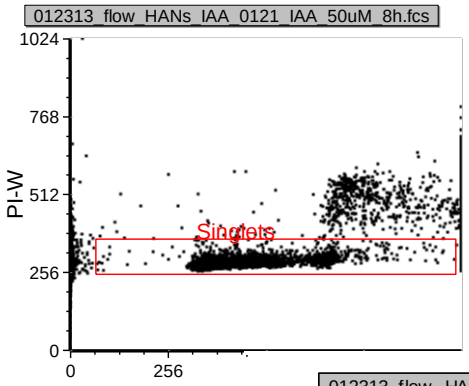
Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=15.3, %G2=40.3
The S Phase confidence is fair
Note: inter-model error,

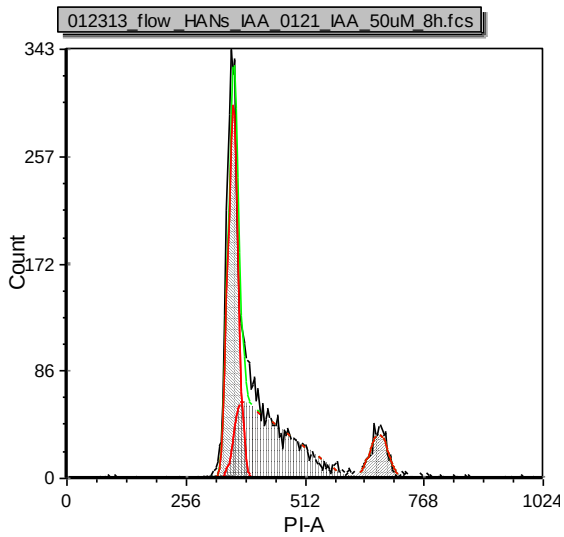
Experiment Statistics

Chi sq: 1.50
BAD: 0.90
Mean Xtra S: 659.37
Number of cells: 4975.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

IAA 50 µM, 8 hr, +S order=1 model, 1 cycle



Model	Dip %G2	Dip %S	Chis
SL S0	7.73	25.92	6.15
SL CL S0	7.16	26.04	5.96
+G2/G1 Fixed	0.00	32.01	6.87
+Aggregates	0.00	31.57	6.91
+S Order = 1	9.40	45.87	1.53
+CVs Fixed	9.43	46.20	1.51



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	358.47	3.09	44.74	672.46	2.90	9.40	45.87	1.88	100.00	0.41

Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=45.9, %G2=9.4
The S Phase confidence is fair
Note: inter-model error,

Experiment Statistics

Chi sq: 1.53
BAD: 0.41
Mean Xtra S: 659.37
Number of cells: 4980.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Appendix D.4 Plating efficiency of HAA-treated CHO cells. The blank-corrected absorbency values were normalized to the concurrent negative control (raw data used for Figure 5.4).

Table D.4 Cell density of negative control CHO cells measured as the blank-corrected absorbency values normalized to the concurrent negative control (%).

	Negative control
	102.636
	104.198
	94.636
	102.340
	100.082
	101.011
	98.119
	96.979
	100.611
	91.680
	104.652
	107.842
	93.381
	89.979
	116.560
	95.295
	98.592
	96.983
	113.075
	95.603
	98.592
	92.155
	97.443
	107.557
number	24
Average	100.000
SE	1.342
SD	6.575

Table D.5 Cell density of IAA-treated CHO cells measured as the blank-corrected absorbency values normalized to the concurrent negative control (%).

	IAA				
	10 uM	15 uM	20 uM	25 uM	30 uM
	104.557	90.055	95.987	108.209	83.574
	105.591	96.113	96.324	103.100	87.543
	106.246	101.433	106.858	120.452	108.019
	115.787	102.045	106.351	108.040	106.225
	97.865	103.058	79.120	104.071	95.965
	108.483	112.494	105.887	103.607	94.002
	101.749	88.007	80.408	103.565	97.063
	109.708	98.034	66.708	61.663	92.060
	89.211	103.016	89.569	91.807	72.492
	95.142	92.335	99.807	103.333	47.309
	100.166	78.973	101.982	85.369	83.553
	109.011	97.654	111.882	104.874	104.747
	99.259	107.639	104.008	99.174	88.155
	105.570	107.533	101.074	93.221	51.995
	106.499	83.152	107.111	100.293	86.825
	95.459	93.538	59.278	60.016	53.261
number	16	16	16	16	16
Average	103.144	97.192	94.522	96.925	84.549
SE	1.673	2.294	3.862	4.022	4.770
SD	6.691	9.178	15.448	16.087	19.081

Table D.6 Cell density of BAA-treated CHO cells measured as the blank-corrected absorbency values normalized to the concurrent negative control (%).

	BAA				
	30 µM	60 µM	90 µM	120 µM	150 µM
	100.399	105.077	58.081	66.800	43.833
	81.685	66.374	46.598	52.339	46.810
	84.024	77.007	42.557	81.260	35.540
	93.381	101.249	57.230	38.729	94.232
	79.771	51.489	41.494	41.281	39.793
	106.353	106.991	46.172	40.005	38.091
	127.406	55.316	52.977	85.938	50.425
	103.801	82.323	53.402	67.012	54.040
	111.882	45.534	75.306	53.615	51.489
	118.049	41.281	40.643	53.828	43.833
	90.191	52.127	41.707	73.179	32.775
	82.323	97.422	62.121	50.425	84.875
	109.755	106.778	81.898	46.598	48.511
	62.759	47.023	41.494	45.534	37.666
	100.611	71.265	45.534	54.040	45.109
	60.207	59.782	53.828	49.575	52.764
number	16	16	16	16	16
Average	94.537	72.940	52.565	56.260	49.987
SE	4.705	6.018	3.065	3.614	4.191
SD	18.822	24.070	12.260	14.457	16.764

Table D.7 Cell density of CAA-treated CHO cells measured as the blank-corrected absorbency values normalized to the concurrent negative control (%).

	CAA				
	2 mM	3 mM	4 mM	6 mM	8 mM
	98.592	96.063	93.305	95.603	93.764
	92.845	94.914	99.971	105.489	76.523
	92.615	84.799	102.730	93.305	82.270
	104.339	104.799	110.086	96.293	88.017
	95.603	96.523	102.500	96.753	87.098
	91.236	110.086	98.132	101.810	73.534
	99.052	117.213	119.741	106.638	94.684
	112.615	98.822	105.259	99.741	81.121
	97.443	102.960	96.063	96.983	80.661
	96.753	102.040	89.626	91.925	79.511
	104.109	105.489	104.799	93.075	78.132
	100.201	89.626	108.247	98.592	71.925
	113.305	115.374	91.006	99.741	88.017
	99.052	109.397	91.236	87.557	76.063
	113.534	104.569	115.144	102.040	76.983
	103.190	104.799	104.109	87.787	71.466
number	16	16	16	16	16
Average	100.905	102.342	101.997	97.083	81.236
SE	1.797	2.178	2.175	1.388	1.810
SD	7.187	8.712	8.700	5.552	7.242

Appendix D.5 Raw data of the DNA strand breaks measured by single cell gel electrophoresis assay. CHO cells were treated with different concentration of HAAs in a 96-well microplate or a 6-well plate for 4 hours. Values shown are the mean % tail DNA of 50 nuclei of each treatment group (raw data used for Figures 5.5, 5.6 and 5.7).

CAA concentration (mM)	%Tail DNA with 96 well	%Tail DNA with 6 well
0	1.5843	0.5038
3	2.4260	0.5367
4	2.6446	3.4106
6	18.8711	4.2083
7	8.3836	4.8530

BAA concentration (µM)	%Tail DNA with 96 well	%Tail DNA with 6 well
0	2.0186	1.3259
40	2.7490	0.9086
60	6.3469	1.0179
80	7.8496	1.1773
100	22.3574	2.4504

IAA concentration (µM)	%Tail DNA with 96 well	%Tail DNA with 6 well
0	1.2938	0.6983
10	6.5756	1.7640
20	19.5038	10.8328
25	22.7087	13.6607
30	26.8348	21.7422